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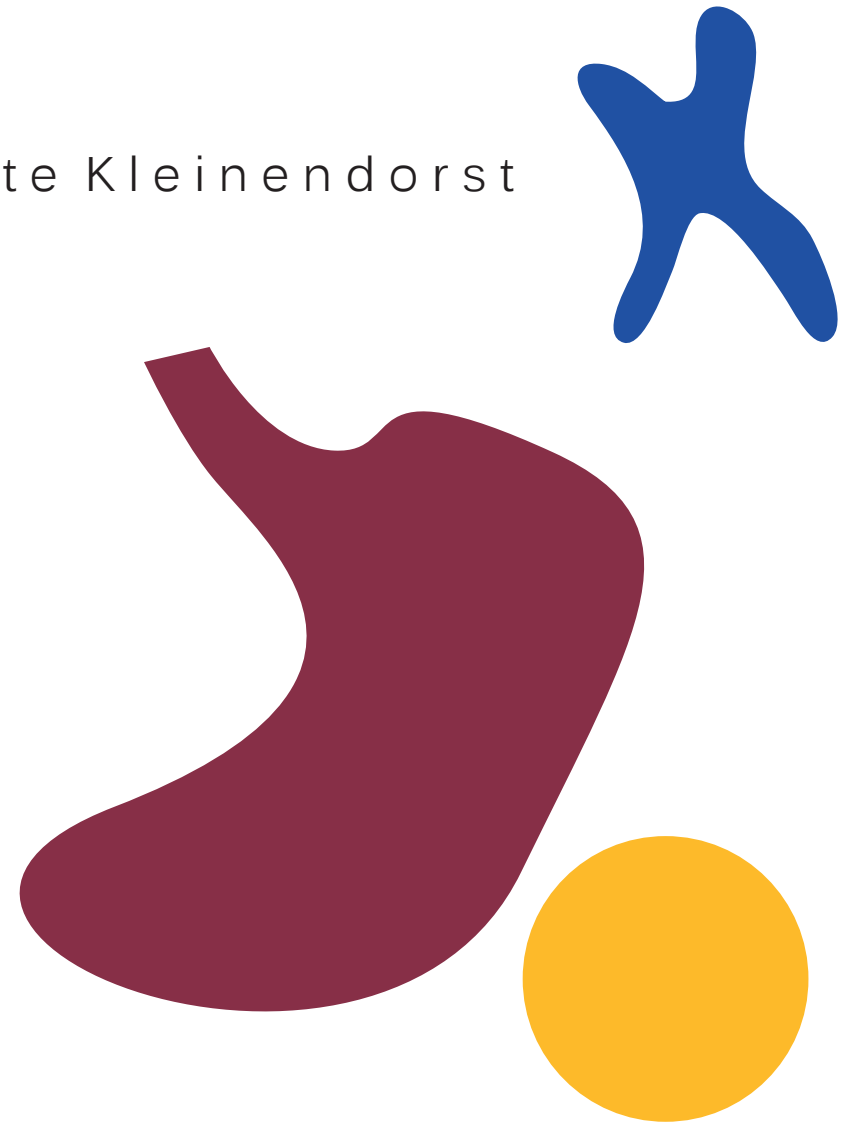
Lotte Kleinendorst



Genetic Obesity: Disorders and Diagnostics

Genetic Obesity: Disorders and Diagnostics

Lotte Kleinendorst

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Genetic Obesity:
Disorders and Diagnostics

ACADEMISCH PROEFSCHRIFT

ter verkrijging van de graad van doctor

aan de Universiteit van Amsterdam

op gezag van de Rector Magnificus

prof. dr. ir. K.I.J. Maex

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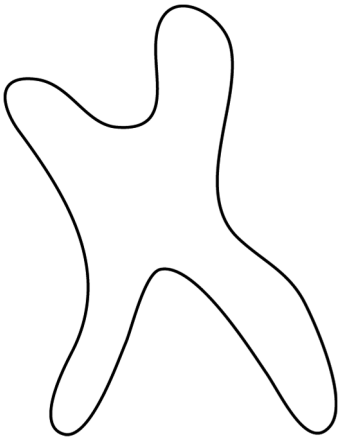
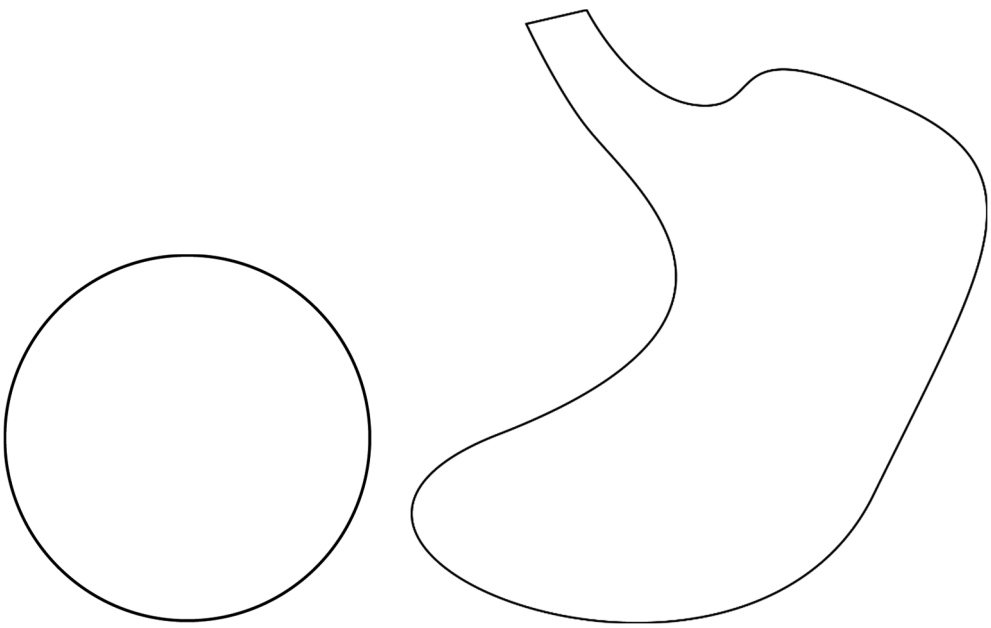
Chapter 1



General introduction and outline of the thesis



Obesity is an accumulation of body fat caused by an imbalance of energy intake and energy output, which leads to an increased health risk. It is one of the most common and serious health problems of our time which can be seen in every health clinic in the world. Worldwide, the prevalence of obesity 12% among adults and 5% among children and adolescents.(1) The prevalence of obesity is increasing at a fast pace, and it is estimated that one-fifth of the world population will have obesity by 2025.(2)

Healthcare professionals determine an individual's weight status by using the body mass index (BMI), the calculation of body weight in relation to square height. Obesity starts at a BMI of 30 kg/m2 (obesity and overweight 2018). In children, BMI standard deviation scores (SDS) are needed to assess overweight and obesity because BMI in children varies greatly while growing. They represent the deviation from the mean BMI in children of the same sex and same age. The health consequences of obesity extend to all organ systems. In the long term, this can result in cardio- and cerebrovascular diseases, cancer, and even premature death.(3) Obesity related medical problems like osteoarthritis and polycystic ovary syndrome have a great impact on the well-being of affected people as well.(3) Moreover, the impact of the stigma and its psychological effects are great threats to the quality of life of people with obesity.(4) Obesity and its related health consequences are therefore a large threat to our societal resources, not only because of high healthcare expenditure but also in productivity loss and reduced well-being.(5)

The human body is an excellent energy battery. When little food is available, the body uses its energy storage; and when resources are superfluous, extra energy is stored in the form of fat. Day-to-day energy consumption and energy expenditure are regulated by a system of complex neurological and endocrine pathways. This balance can be easily disrupted in our current environment, with numerous external factors that can lead to obesity. The causes of obesity are diverse, but the recent rise of its prevalence is mainly due to change in our environment.(6) Our society has become more obesogenic with easily accessible energy-dense food and a reduction in physical activity. Other factors such as sleep, stress, and medication can play a role in developing obesity as well. Somatic disorders such as Cushing's syndrome or hypothalamic damage are rare causes of obesity. It is well known that environmental circumstances, such as sedentary lifestyle and fast-food consumption, are important players in the development of obesity, but variation in weight or BMI is also highly attributable to the genetic background. Multiple twin studies led to the conclusion that the heritability of weight can be as high as 70%.(7) In many people, obesity predisposition is probably polygenic and multifactorial, meaning that the combination of environmental factors and different genetic factors determine body weight. Currently, more than 100 genes are identified to be associated with obesity or BMI.(8) There are far less genes in which a single mutation leads to obesity regardless of environmental factors.

Genetic obesity disorders

For a small percentage of people with obesity a genetic defect is the main cause of their obesity. Genetic obesity is often divided into syndromic obesity and non-syndromic obesity. Syndromic obesity is the name used for genetic obesity disorders with intellectual disability/developmental delay, congenital anomalies, and/or organ dysfunction. There are however genetic disorders in which obesity is the main symptom; they are often called non-syndromic obesity disorders. Here we briefly introduce the most important non-syndromic and syndromic obesity disorders that are discussed further in this thesis.

Genetic obesity disorders without intellectual disability (non-syndromic)

1. LEP and *LEPR*

Congenital leptin deficiency was first identified in a consanguineous family with two obese cousins from Pakistan. These patients had very low leptin levels in serum and suffered from early-onset morbid obesity. A homozygous frameshift mutation in LEP was identified in both children.(9) The first patient with LEPR deficiency was identified in a consanguineous family as well.(10) These patients have severe hyperphagia (abnormally increased appetite with decreased satiety). Leptin and leptin receptor deficiency are thought to be very rare.

2. MC4R

Melanocortin-4-receptor deficiency is the most common genetic form of obesity. In 1998 two independent research groups identified the first patients with MC4R deficiency at the same time, which led to a back-to-back publication of the articles “A frameshift mutation in *MC4R* associated with dominantly inherited human obesity” and “A frameshift mutation in human *MC4R* is associated with a dominant form of obesity” in Nature Genetics.(11, 12) MC4R is a G-protein-coupled receptor, a class of receptor that is crucial for signal transduction pathways, and therefore one of the most important drug targets in modern medicine. *MC4R* is a main player in the leptin-melanocortin pathway.

3. POMC

Autosomal-recessive mutations in the proopiomelanocortin (*POMC*) gene were first detected in 1998, in two nonrelated patients with morbid obesity and adrenal dysfunction.(13) Their striking phenotype included pale skin and red hair. This can be explained by the cleavage of the POMC protein into different peptides. One of these peptides, α-MSH is needed for the production of skin and hair pigments. Deficiency of the second cleave product, adrenocorticotropic hormone (ACTH) results in adrenal dysfunction.

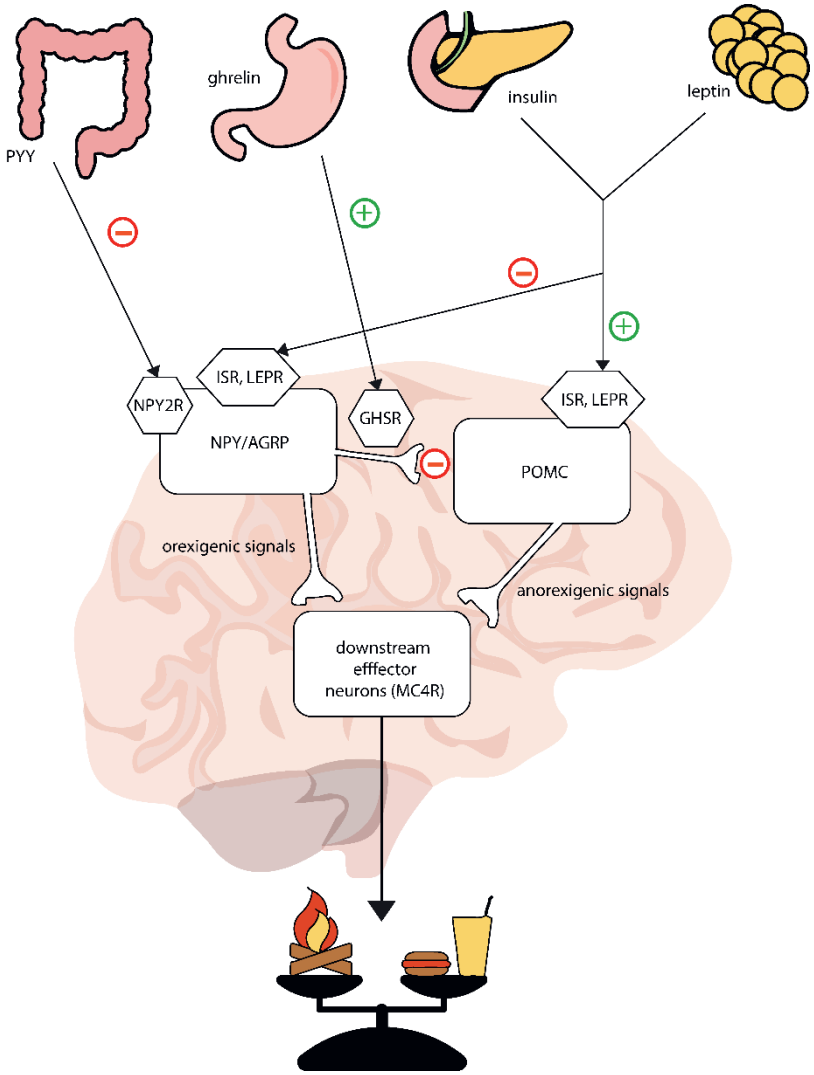


Figure 1.
A schematic overview of the different players in the leptin-melanocortin pathway leading to changes in energy expenditure and food intake through several down-stream effector neurons including MC4R. In the well-fed situation, leptin is secreted from adipose tissue. Leptin binding to its receptor has several effects, including proopiomelanocortin (POMC) production. POMC is then cleaved into the melanocortins α- and β-MSH. This binds to the melanocortin 4 receptor (MC4R). MC4R activity will lead to decreased food intake and increased energy expenditure.
AGRP = agouti-related peptide, GHSR = growth hormone secretagogue receptor, ISR = insulin receptor, LEPR = leptin receptor, MC4R = melanocortin 4 receptor NPY = neuropeptide Y, NPY2R = neuropeptide Y 2 receptor, POMC = proopiomelanocortin PYY = Peptide YY

Syndromic obesity disorders

1. Prader-Willi syndrome

Prader-Willi syndrome (PWS) is the best known syndromic obesity disorder and is caused by genetic changes within the Prader-Willi critical region on the long arm (q) of chromosome 15. The time line of the weight gain in PWS is very typical for the disease: from the neonatal period till the first year of life, a baby with PWS is hypotonic and has severe feeding difficulties due to poor suck and reduced appetite, for which tube feeding is almost always required. From the age of 1 year the children show an improved appetite and weight increase. The extreme hyperphagic phase that is typical for PWS often starts around the age of 8.(14) The exact mechanism of obesity in PWS is unclear, but a hypothalamic defect is most likely the cause of the lack of satiety in children with PWS. Moreover, the neonatal phase with feeding problems could cause a long-term decreased caloric requirement in children with PWS.

2. Bardet-Biedl syndrome

Another well-known genetic obesity syndrome is Bardet-Biedl syndrome (BBS), a ciliopathy disorder characterized by intellectual deficit, polydactyly, rod-cone dystrophy, and renal problems. There are more than 20 genes associated with BBS. Most of the patients, between 72% and 92%, have obesity.(15) Their weight gain often starts in the first year of life. Leptin resistance is found in patients with BBS, similar to other patients with obesity. Since BBS is a disease of cilia dysfunction, it makes sense that cilia also play a role in the leptin-melanocortin pathway. Murine studies showed that the BBSome interacts with the LepR.(16) Besides this, BBS patients show less physical activity than healthy controls.(17) Obesity in BBS is therefore probably caused by both increased caloric intake due to leptin resistance and lower energy expenditure.

3. 16p11.2 deletion

There are several copy number variations associated with obesity. The most common is a 650 kb deletion on chromosome 16p11.2. There is a large variation in symptoms and severity of symptoms among patients with a 16p11.2 deletion. Most patients have mild intellectual disability. The delay in language development is often more severe than the motoric development. Behavioral problems are frequently described, including autism and attention deficit hyperactivity disorder (ADHD). Affected patients also have macrocephaly. The obesity phenotype in patients with a 16p11.2 deletion occurs later in childhood than in patients with mutations in the leptin-melanocortin pathway genes. Interestingly, individuals with a duplication at 16p11.2 show a mirror phenotype with microcephaly and an increased risk of being underweight.(18) The 16p11.2 region includes around 25 genes. One gene in the 16p11.2 deletion that might be responsible for the obesity phenotype is *SH2B1*. Patients with a deletion including only *SH2B1* or patients with mutations in *SH2B1* are more likely to overeat and have rapid weight gain and insulin resistance.(19) *SH2B1* plays a supporting

role in the leptin-melanocortin pathway, possibly because it enhances the leptin sensitivity of the hypothalamus.(20)

Diagnosing genetic obesity disorders

The current international guideline for obesity in children and adolescents of the Endocrine Society suggests genetic testing in children with extreme early-onset obesity before the age of 5 years.(21) Previously, genetic testing for specific obesity syndromes was only available as single-gene Sanger sequencing. Nowadays, with gene panel analysis or whole exome sequencing, multiple genes can be analyzed at the same time. But not all obesity syndromes can be identified using these techniques, for example, imprinting disorders like Prader-Willi syndrome or Temple syndrome request specific DNA methylation-specific testing. In patients with obesity and intellectual disability or developmental delay, one can also consider to perform chromosomal microarray analysis.

Treating genetic obesity disorders

A genetic diagnosis facilitates personalized medical treatment and expert healthcare. It can also support decision-making in bariatric surgery and lead to specific drug treatment. In general, it is difficult to achieve long-term weight loss.(22) When people reduce their calorie intake, metabolism slows down and less calories are burnt.(23) To lose more weight, a person has to decrease intake even more. Low-calorie diets also lead to changes in satiety hormones. One year after weight loss, the circulating levels of ghrelin are still higher than before weight loss and the levels of satiety hormones like leptin and PYY are lower, as if the body tries to regain its old weight.(24) Since patients with genetic obesity disorders are often hyperphagic, food access restriction seems the most promising prevention method or treatment strategy for them. Whether this is feasible depends on environmental circumstances as well. To date, there are no therapies to cure the primary causes of genetic obesity disorders. Leptin is a satiety hormone for which replacement therapy is available. Recombinant human leptin was first tested in a trial which included one patient, a 9-year-old girl with congenital leptin deficiency.(25) The therapy is highly efficient in achieving weight loss (by reducing hyperphagia and increasing energy expenditure) and also in correcting the hormonal abnormalities in leptin-deficient patients. Leptin therapy is unfortunately not effective to treat other types of obesity, because most people with obesity are leptin resistant. New drugs are developed to treat common obesity or rare genetic obesity disorders, among which are melanocortin 4 receptor agonists. Since MC4R agonists replace MSH, they can be effective in several disorders with a defective leptin-melanocortin pathway. Clinical trials in POMC deficiency and LEPR deficiency have shown impressive reductions of hyperphagia and body weight.(26, 27) Several other new pharmacological agents might be useful in patients with genetic obesity as well, for example, analogs of GLP1, which decreases appetite.(28) In the general population, bariatric surgery is the most effective obesity treatment option. Whether this is as effective

for genetic obesity as well is unsure, especially because data on follow-up are lacking for most disorders. It is known that gastric bypass surgery leads to changes in gut hormones that influence satiety. Patients with heterozygous *MC4R* mutations who underwent a Roux-en-Y gastric bypass seem to have similar short-term follow-up results as patients without *MC4R* mutations.(29) Last but not the least, genetic counseling is a part of treatment for genetic obesity that should not be overlooked. Establishing a genetic diagnosis can end the "diagnostic odyssey," the journey that patients and their families have to undertake to reach an etiological diagnosis. A genetic diagnosis can reduce the obesity stigma, giving insight that obesity is not only a matter of poor lifestyle choices and little willpower. Establishing a genetic diagnosis may also support reproductive decision-making and help early intervention in other family members.

This general introduction is based on excerpts from our two book chapters:

- Genetics of Obesity. Kleinendorst L, van Haelst MM, van den Akker ELT. In: Igaz P, Patócs A, editors. Genetics of Endocrine Diseases and Syndromes. Cham: Springer International Publishing; 2019. p. 419-41.
- Molecular basis of obesity disorders, Lotte Kleinendorst, Mieke M. van Haelst. In: Dhavendra Kumar, editor. Clinical Molecular Medicine, Academic Press, 2020, p. 73-88.

Outline of the thesis

The content of this thesis focuses on a wide range of genetic obesity disorders and the clinical approach to diagnose them. The content of this thesis is divided in three parts. The **first part** focuses on the genotype and diagnostics of genetic obesity disorders. We discuss the diagnostic yield of our next-generation sequencing gene panel for obesity in two large cohorts. The **second part** of the thesis zooms in on the phenotype of the patients with genetic obesity disorders. We performed one of the first studies which systematically examines the underlying medical causes of obesity in a pediatric obesity cohort. With this broad diagnostic approach, we hope to improve the identification of rare genetic obesity disorders. In the next chapter of this part, we study one specific genetic obesity disorder, namely leptin receptor deficiency. We give an overview of the phenotype, both from literature and from our own clinic, and try to estimate the prevalence of this relatively unknown disorder. The **third part** focuses on the implications of diagnosing a genetic obesity disorder. Case reports and a qualitative study illustrate the impact of these disorders on the patients and their parents.

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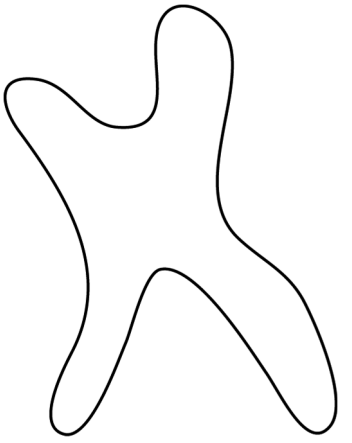
Part I

DNA DIAGNOSTICS FOR RARE GENETIC OBESITY DISORDERS

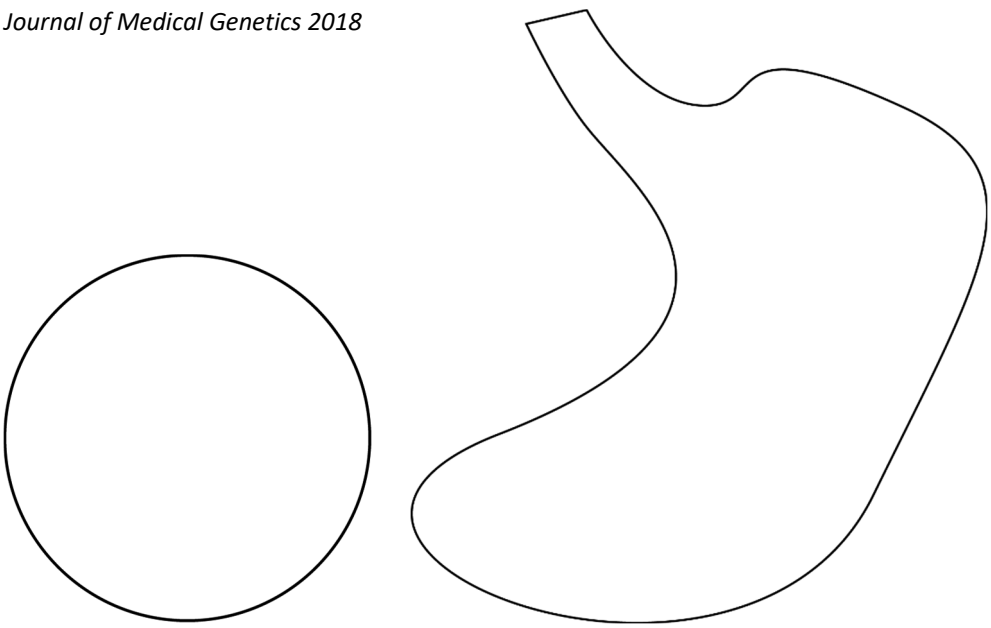
Chapter 2



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

L. Kleinendorst, M.P.G. Massink, M.I. Cooiman, Mesut Savas, O.H. van der Baan-Slootweg, R.J. Roelants, I.C.M. Janssen, H.J. Meijers-Heijboer, N.V.A.M. Knoers, J.K. Ploos van Amstel, E.F.C. van Rossum, E.L.T. van den Akker, G. van Haaften, B. van der Zwaag, M.M. van Haelst

Journal of Medical Genetics 2018



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/m2.1 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), can severely affect this energy balance. (2) 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. 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.

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:

1. Age of onset of obesity <5 years (prepubertal onset in adult subgroups)
2. Family history of obesity (alarm symptom: single person with obesity in family)
3. Hyperphagia
4. Intellectual deficit/developmental delay
5. Congenital malformations
6. Visual impairment and/or deafness
7. 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

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.

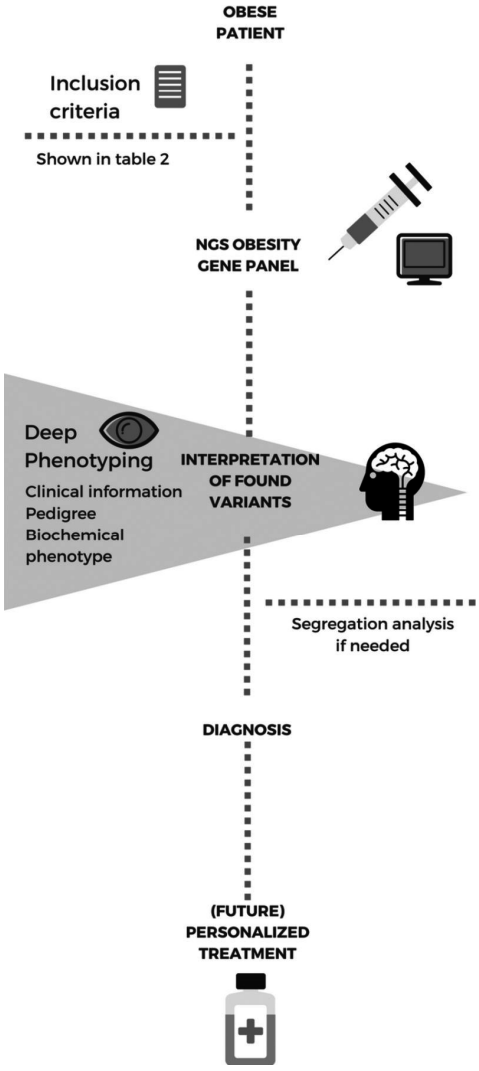


Figure 1. Diagnostic process

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.

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/m2 (lowest 22, 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.

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

Pt	Age (yrs)	Gender	Medical Center	Gene	Genotype	MCA/ ID	Clinical Information Fitting with the clinical phenotype	Additional information / Coincidental phenotypic findings	BMI (kg/m^2)	SD (children)	Family history 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	+6·1SD	
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	+7·5SD	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 grams (-0.4SD for gestational age)	Cleidocranial dysplasia (*RUNX2* mutation identified)	34·6	+7·3SD	No family history of obesity
4	17	M	2	*MC4 R*	**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	*MC4 R*	**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 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	+3·7SD	Mother and brother obese

Medical center: 1=UMC Utrecht Clinical Genetics; 2=CGG Pediatrics. **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 analyzed is not suitable for reliable detection of genomic deletions >6 basepairs.

Table 2. Confirmed diagnosis genetic obesity: autosomal dominant inheritance

Pt	Age (yrs)	Gender	Medical Center	Gene	Genotype	MCA/ ID	Clinical Information Fitting with the clinical phenotype	Additional information /Coincidental phenotypic findings	BMI (kg/ m^2)	BMI-SD	Family history obesity
7	2	M	3	*GNAS*	**Heterozygous:** c.85C>T; p.(Gln29*)	Yes	Developmental delay Hyperphagia Onset obesity: 1·5 years		21·4	+2·9SD	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	+2·4SD	
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		50·5	-	

							duodenal-ilial bypass Onset obesity: 25 years				
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 obese brother
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	+38SD	Mother obese, no segregation analysis performed
14	15	F	2	*MC4R*	**Heterozygous:** c.105C>A; p.(Tyr35*)	No	Onset obesity: 9 years		36·8	+3SD	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	+2·7SD	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	+4·4SD	
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 obese daughter, 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 492kb 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	+5·3SD	
22	7	F	10	*MC4R*	**Heterozygous:** c.240C>A; p.(Tyr80*)	No	Onset obesity: 5 years		28·6	+4SD	
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	+2·9SD	
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 (+1,5SD)	SNP-array XXY syndrome	25·3	+5SD	No family history of obesity, *de novo* mutation

							Head circumference 56 cm (+2,5SD)				
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	+4·5SD	Both parents overweight
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	+3·9SD	The *PCSK1* mutation was identified in the obese mother
32	13	F	5	*POMC*	**Heterozygous:** c.206del; p.(Pro69fs)	Yes	Onset obesity: 3 years	Special education Sacral dimple	43	+4·3SD	The *POMC* mutation was identified in the obese mother
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	44·1	-	

								(*SLC26A4*: neg.)			
36	14	M	5	POMC	**Heterozygous:** c.662A>G; p.(Tyr221Cys)	No	Onset obesity: 8 years		35·8	+3·8SD	
37	60	M	4	*POMC*	**Heterozygous:** c.662A>G; p.(Tyr221Cys)	No	Onset obesity: 8 years	Myopia (-8Dpt)	39·3	-	
38	6	F	1	*POMC*	**Heterozygous:** c.706C>G; p.(Arg236Gly)	No	Onset obesity: 3 years		26	+4SD	The *POMC* mutation was identified in the obese mother
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 (+2·4SD)	57	-	The *POMC* mutation was **not** identified in the obese mother
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	+3·8SD	
47	5	F	1	*SIM1*	**Heterozygous:** c.875_877del; p.(Thr292del)	No	IQ 71 Onset obesity: 2·5 years	20·8	+3SD	
48	46	F	4	*SIM1*	**Heterozygous:** c.1532del;p.(Asn 511fs)	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 in patients with a genetic obesity disorder

The median BMI in adult patients with a definitive diagnosis was 41.8 kg/m2 (range 34.2–72.7). Patients without a definitive or likely diagnosis had a median BMI of 43.7 kg/m2 (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 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).

<u>Illustrative cases and (future) personalised treatment</u>

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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Footnote

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SUPPLEMENTARY APPENDIX

1. Patient subgroups and characteristics

Adult subgroups

1. *Vitalys, Center for Bariatric Surgery*
   The bariatric surgery cohort (659 patients) was recruited at the Vitalys center for bariatric surgery in the Rijnstate Hospital, Velp, The Netherlands. All participants underwent bariatric surgery and received diagnostic gene panel analysis.

2. *Obesity Center CGG (Centrum Gezond Gewicht), Erasmus MC, University Medical Center Rotterdam: Adult division*
   The Obesity Center CGG is a collaboration between Erasmus University Medical Center, Sint Franciscus Hospital and Maasstad Hospital in Rotterdam, The Netherlands. In this center extensive diagnostics of adults and children with obesity is performed, aiming to provide personalized obesity treatments in a multidisciplinary approach. Diagnostic gene panel analysis was performed for 124 adult patients in this center.

Pediatric subgroups

3. *Obesity Center CGG (Centrum Gezond Gewicht), Erasmus MC, University Medical Center Rotterdam: Pediatric division*
   Diagnostic gene panel analysis was performed for 94 children with severe early onset obesity who were referred to this tertiary center by medical specialists for diagnostics of possible underlying causes.
4. *VUmc Louwesweg*
   The pediatric department of the VUmc location Louwesweg is an outpatient clinic in Amsterdam, providing secondary care for patients from the Amsterdam region. Most obese patients seen in this clinic have lifestyle related obesity. Diagnostic gene panel analysis was performed for 44 patients at the pediatric department of the VUmc location Louwesweg.
5. *Childhood Obesity Center Heideheuvel*
   Heideheuvel is a specialized obesity clinic for children with extreme therapy resistant obesity, where combined lifestyle intervention is offered in both ambulatory and inpatient treatment programs. Diagnostic gene panel analysis was performed for 26 patients in this center.

2. Supplementary Methods

Statistical analysis

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. Distributions of the BMI for with and without diagnosis were similar, as assessed by visual inspection. Median BMI was not statistically significantly different between with and without diagnosis, U = 9.230, z = 1.057, p = .698. The same test was performed to determine if there were differences in BMI-SD in children between those with a diagnosis and without a definite diagnosis. Distributions of the BMI-SD were similar, as assessed by visual inspection. Median BMI-SD was not statistically significantly different between with and without diagnosis, U = 960, z = 145, p = .280.

Bardet-Biedl Syndrome mutations analysis

We determined the population allele frequencies for a set of 27 curated pathogenic *BBS* gene mutations (Table S3) in the obesity gene panel cohort. We excluded the 48 definitive diagnosed cases (Tables 2 and 3) for this analysis and compared against the Non-Finnish European (NFE) population of the ExAC database (http://exac.broadinstitute.org/). We show a 1.7 fold higher population allele frequency for *BBS* mutation carriers in the obesity gene panel cohort compared to the ExAC's NFE population (Table S3). To determine the significance of this result we performed permutation testing on the obesity gene panel and ExAC NFE populations allele frequency data. For this we used the frequency data for filtered variants of all 255 genes present on the obesity gene panel (52 diagnostic genes from the official gene panel described in this paper and the extra not yet analyzed 203 research genes). The variants used in permutation testing are filtered on the following criteria: read_depth ≥ 20, population frequency < 1% in 1000Genomes, ESP6500 and GoNL databases, and the variants must have a functional effect on the protein level (nonsynonymous, frameshift, stopgain, stoploss or startloss) or affect a canonical splice site sequence (+/- 2bp). Furthermore, only heterozygous variants are used in the analysis. For permutation testing, the population allele frequency fold difference between the obesity gene panel and ExAC NFE populations are determined for n=100.000 random sets of 27 variants. The number of permutations with an equal or higher fold difference than 1.7 is divided by the total number of permutations and determines the significance score. The resulting 81310/100000 permutation score is not statistically significant.

3. Supplementary Tables

Table S1: Variants of uncertain clinical significance (VUS) identified in obese patients, that could possibly lead to a diagnosis in the future.

Further segregation analysis and/or functional studies will be performed to elucidate the pathogenicity of these variants.

Pt	Age	Gender	Gene	Genotype	Mode of inheritance	Other mutations found	Result segregation analysis (if performed)
49	14	F	***BBS9***	c.2258A>T;p.(Glu753Val);c.310del; p.(Cys104fs)	AR		
50	48	F	***BDNF***	c.133A>C; p.(Ser45Arg)	AD		Overweight mother carrier
51	39	M	***BDNF***	c.440G>A; p.(Trp147*)	AD		Obese daughter carrier, non-obese mother carrier
52	21	F	***CEP290***	c.6516del; p.(Lys2172fs)(Pathogenic); c.564T>G; p.(Asp188Glu)}	AR		Non obese parents carriers
53	11	M	***CEP290***	c.2217+3G>C;p.(?); c.2980G>A;p.(Glu994Lys)	AR		
54	52	F	***CRHR2***	c.650G>T; p.(Gly217val)	AD		
55	25	M	***CRHR2***	c.842G>A; p.(Arg281His)	AD		Overweight mother carrier
56	50	F	***FLOT1***	c.43G>A; p.(Gly15Arg)	AD		
57	8	F	***G6PC***	c.980_982del; p.(Phe327del)	AD		
58	30	F	***G6PC***	c.508C>T; p.(Arg170*)	AD	Pathogenic IRS1 VUS	
59	47	F	***G6PC***	c.1039C>T; p.(Gln347*)	AD		
60	10	F	***KIDINS220***	c.603+3_603+7del; p.(?)	AD		Obese mother carrier
61	4	F	***KIDINS220***	c.5242G>T; p.(Asp1748Tyr)	AD	MKS1 VUS	
62	56	F	***KIDINS220***	c.2635A>G p.(Arg879Gly)	AD		Non-obese daughter carrier
63	43	F	***KIDINS220***	c.5221A>T; p.(Ser1741Cys)	AD		
64	42	F	***KIDINS220***	c.207+3A>G; p.(?)	AD		
65	9	M	***KIDINS220***	c.1117C>T; p.(His373Tyr)	AD		
66	55	F	***KIDINS220***	c.3139T>C; p.(Phe1047Leu)	AD		

67	30	F	***KIDINS 220***	c.5002C>T; p.(Pro1668Ser)	AD		Obese mother non-carrier
68	6	F	***LEPR***	c.2260G>A; p.(Val754Met)	AD	CEP290 VUS	
69	46	F	***LEPR***	c.1717C>A; p.(Arg573Ser); c.1717C>A; p.(Arg573Ser)	AR		
70	60	F	***LEPR***	c.1835G>A; p.(Arg612His)	AD		
71	10	F	***MC3R***	c.950G>T; p.(Cys317Phe)	AD		Overweight mother carrier
72	11	M	***MCHR 1***	c.950G>A; p.(Arg317Gln)	AD		Non-obese father carrier
73	44	F	***MCHR 1***	c.950G>A; p.(Arg317Gln)	AD		
74	4	M	***MCHR 1***	c.950G>A; p.(Arg317Gln)	AD		Obese mother and grandmother carriers
75	8	M	***MCHR 1***	c.790G>A; p.(Gly264Ser)	AD		
76	24	F	***MCHR 1***	c.950G>A; p.(Arg317Gln)	AD	Pathogenic TMEM67	Obese mother carrier MCHR1
77	3	F	***MCHR 1***	c.950G>A; p.(Arg317Gln)	AD		Non-obese father carrier
78	64	M	***MCHR 1***	c.950G>A; p.(Arg317Gln)	AD		
79	42	F	***MCHR 1***	c.950G>A; p.(Arg317Gln)	AD		
80	14	F	***MCHR 1***	c.950G>A; p.(Arg317Gln)	AD		
81	21	F	***MCHR 1***	c.694G>C; p.(Val232Leu)	AD		
82	13	F	***MCHR 1***	c.950G>A; p.(Arg317Gln)	AD		Obese mother carrier
83	5	F	***MRAP 2***	c.499C>G; p.(Pro167Ala)	AD		
84	34	F	***MRAP 2***	c.373C>T; p.(Arg125Cys)	AD	Pathogenic CEP290	
85	47	F	***MRAP 2***	c.373C>T; p.(Arg125Cys)	AD		
86	18	F	***MRAP 2***	c.373C>T; p.(Arg125Cys)	AD		Obese mother non-carrier
87	50	F	***MRAP 2***	c.373C>T; p.(Arg125Cys)	AD		
88	54	F	***MRAP 2***	c.373C>T; p.(Arg125Cys)	AD		
80	15	M	***NDN***	c.694C>G; p.(Arg232Gly); (VUS)	AD	BBS2 VUS	
90	28	M	***NTRK2***	c.500C>A; p.(Ser167Tyr)	AD		
91	51	F	***NTRK2***	c.881C>T; p.(Ser294Phe)	AD		Non-obese brother non-carrier

92	59	M	***PCK1***	c.1397C>T; p.(Ala466Val); c.1628G>A; p.(Arg543Gln)	AR		Non-obese brother non-carrier
93	10	F	***PCSK1***	c.934C>T; p.(Arg312Cys)	AD	CEP290 VUS	
94	14	F	***PCSK1***	c.736A>G; p.(Thr246Ala)	AD		
95	53	F	***PCSK1***	c.539A>G; p.(Asn180Ser)	AD	Pathogenic MC3R; IRS1 VUS	
96	44	F	***PCSK1***	c.1991C>T; p.(Ser664Phe)	AD		Obese sister non-carrier
97	28	F	***PCSK1***	c.1991C>T; p.(Ser664Phe)	AD		
98	10	F	***PCSK1***	c.239G>A; p.(Arg80Gln)	AD		
99	63	F	***PCSK1***	c.328C>T; p.(Arg110Cys)	AD		
100	9	F	***POMC***	c.167C>A; p.(Ala56Asp)	AD		Non-obese father carrier
101	6	M	***POMC***	c.394C>G; p.(Pro132Ala)	AD		
102	13	M	***POMC***	c.641A>G; p.(Glu214Gly)	AD	PTEN VUS: c.400A>G; p.(Met134 Val)	
103	10	M	***POMC***	c.285_286ins36; p.(?)	AD		
104	41	M	***POMC***	c.229T>G; p.(Tyr77Asp)	AD		
105	6	F	***POMC***	c.176C>T; p.(Pro59Leu)	AD		
106	47	F	***SIM1***	c.457+9G>A; p.(?)	AD		
107	4	F	***SIM1***	c.1121G>A; p.(Arg374Gln)	AD		Non-obese father carrier
108	6	M	***SIM1***	c.280G>A; p.(Val94Met)	AD		
109	52	M	***SIM1***	c.2144G>T; p.(Gly715Val)	AD		
110	45	F	***SIM1***	c.1649G>A; p.(Arg550His)	AD		
111	43	F	***SNRPN***	c.193C>T;193C>T p.(Arg65Trp);(Arg65 Trp)	AR		
112	52	F	***SNRPN***	c.182G>A; p.(Arg61His)	AD		
113	33	F	***TBX3***	c.2177G>T; p.(Arg726Leu)	AD		
114	35	F	***TBX3***	c.692A>G; p.(Gln231Arg)	AD		Obese mother carrier
115	16	F	***TMEM 67***	c.149_152dup; p.(Glu52fs); c.91dup; p.(Tyr31fs)	AR		

Table S2: Sequence variants identified in comorbidity genes

Pt	Age	Gender	Gene	Genotype	Pathogenic	Diabetes/ thyroid hormone resistance	Other mutations found
58*	30	F	***IRS1***	c.1699C>T; p.(Arg567*)	Yes	No	G6PC VUS
124	5	F	***IRS1***	c.2674A>G; p.(Ser892Gly)	Yes	Unknown	No
125	50	F	***IRS1***	c.2674A>G; p.(Ser892Gly)	Yes	No	No
126	53	F	***IRS1***	c.2674A>G; p.(Ser892Gly)	Yes	No	No
127	47	F	***IRS1***	c.2674A>G; p.(Ser892Gly)	Yes	Yes	No
128	30	M	***IRS1***	c.938C>T; p.(Pro313Leu)	VUS	No	No
129	56	F	***IRS1***	c.955G>A; p.(Gly319Arg)	VUS	No	No
130	4	F	***IRS1***	c.1103C>A; p.(Pro368Gln)	VUS	Unknown	No
131	14	F	***IRS1***	c.1283C>T; p.(Ser428Leu)	VUS	Unknown	No
132	8	F	***IRS1***	c.1283C>T; p.(Ser428Leu)	VUS	Unknown	MKS1 VUS
133	28	F	***IRS1***	c.1684G>A; p.(Gly562Ser)	VUS	No	No
134	14	M	***IRS1***	c.2057_2059del ; p.(Ser686del)	VUS	Unknown	BBS4 pathogenic mutation
11*	52	F	***IRS1***	c.2204T>A; p.(Met735Lys)	VUS	No	Pathogenic MC3R
135	44	F	***IRS1***	c.2560C>T; p.(Arg854Cys)	VUS	No	No
136	4	M	***IRS1***	c.3241C>T; p.(Arg1081Cys)	VUS	Unknown	No
137	20	F	***IRS2***	c.319G>T; p.(Ala107Ser)	VUS	No	No
138	49	F	***THRB***	c.1190A>G; p.(Asp397Gly)	VUS	No	No

* Patient 11 is also listed in Table 3 with a (confirmed diagnosis of genetic obesity (autosomal dominant inheritance); patient 58 is also listed in Supplementary Table 1 (VUS that could possibly lead to a diagnosis in the future).

Table S3: Curated list of BBS gene mutation frequencies in the Obesitome and ExAC NFE populations

Genes and variants*	#Patients with mutation	Gene panel frequency	Frequency in ExAC
ARL6			
Chr3:g.97487043C>T	1	0.00041876	0
Chr3:g.97516872_97516876dup	2	0.000837521	0

BBS1			
Chr11:g.66293652T>G	3	0.001256281	0.001483826
Chr11:g.66298461_66298463del	1	0.00041876	0
BBS10			
Chr12:g.76741494dup	3	0.001256281	0.000677541
BBS12			
Chr4:g.123664162_123664163del	2	0.000837521	5.78016E-05
BBS9			
Chr7:g.33312753C>T	1	0.00041876	8.23805E-06
Chr7:g.33407475G>A	1	0.00041876	0
Chr7:g.33423365_33423368del	1	0.00041876	5.55638E-05
CEP290			
Chr12:g.88454613del	1	0.00041876	0
Chr12:g.88471122C>G	1	0.00041876	0
Chr12:g.88477713T>G	1	0.00041876	0.000107488
Chr12:g.88479860G>A	1	0.00041876	2.7047E-05
Chr12:g.88483242del	1	0.00041876	0
Chr12:g.88487681dup	4	0.001675042	0
MKKS			
Chr20:g.10393446_10393447dup	1	0.00041876	0
Chr20:g.10394053T>C	2	0.000837521	5.76882E-05
MKS1			
Chr17:g.56290344T>C	5	0.002093802	0.000529863
Chr17:g.56293449C>T	2	0.000837521	0.000190445
TMEM67			
Chr8:g.94772149dup	1	0.00041876	0.006409619
Chr8:g.94772207_94772210dup	1	0.00041876	0
Chr8:g.94777845A>T	2	0.000837521	0.000140183
Chr8:g.94792861T>C	1	0.00041876	9.88452E-05
Chr8:g.94803512G>T	1	0.00041876	0
Chr8:g.94811986G>A	5	0.002093802	0.001870561
Chr8:g.94821126T>C	2	0.000837521	3.30267E-05
TTC8			
Chr14:g.89310195G>A	1	0.00041876	1.5706E-05
SUM	**48**	**0.020100503**	**0.011763442**

*Genomic positions, aligned to genome build HG19

Table S4: Results of the segregation analysis for 27 patients with proven pathogenic *BBS* gene mutations

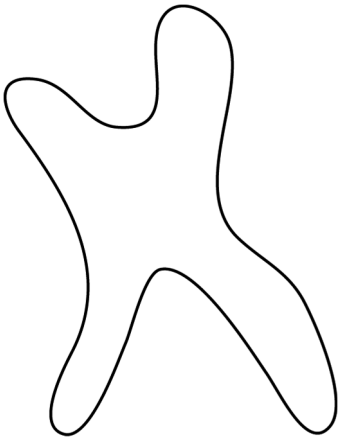
Gene	Geno-type	chro moso me	Genotype	Segregation analysis perfomed	Result
ARL6	M / N	Chr3	97516872_97516876dup	Yes	Non-obese father carrier
ARL6	M / N	Chr3	97516872_97516876dup	No	
ARL6	M / N	Chr3	97487043C>T	No	
BBS1	M / N	Chr11	66293652T>G	No	
BBS1	M / N	Chr11	66293652T>G	Yes	Overweight mother carrier
BBS1	M / N	Chr11	66298461_66298463del	Yes	Obese father carrier
BBS1	M / N	Chr11	66293652T>G	No	
BBS10	M / N	Chr12	76741494dup	Yes	Obese mother carrier
BBS10	M / N	Chr12	76741494dup	No	
BBS10	M / N	Chr12	76741494dup	No	
BBS12	M / PO	Chr4	123664162_123664163del	No	
BBS12	M / PO	Chr4	123664162_123664163del	No	
BBS9	M / N	Chr7	33407475G>A	No	
BBS9	M / N	Chr7	33312753C>T	No	
BBS9	M / N	Chr7	33423365_33423368del	No	
CEP290	M / N	Chr12	88487681dup	No	
CEP290	M / N	Chr12	88487681dup	No	
CEP290	M / N	Chr12	88477713T>G	Yes	Obese mother carrier
CEP290	M / N	Chr12	88454613del	Yes	Non-obese mother carrier
CEP290	M / N	Chr12	88487681dup	Yes	Overweight mother carrier
CEP290	M / N	Chr12	88479860G>A	No	
CEP290	M / N	Chr12	88487681dup	No	
CEP290	M / N	Chr12	88471122C>G	No	
CEP290	M / N	Chr12	88483242del	No	
MKKS	M / N	Chr20	10394053T>C	No	
MKKS	M / N	Chr20	10393446_10393447dup	No	
MKKS	M / N	Chr20	10394053T>C	Yes	Obese father carrier
MKS1	M / N	Chr17	56290344T>C	No	
MKS1	M / N	Chr17	56290344T>C	No	
MKS1	M / N	Chr17	56293449C>T	Yes	Non-obese niece carrier; obese niece non-carrier

MKS1	M / N	Chr17	56293449C>T	No	
MKS1	M / PO	Chr17	56290344T>C	No	
MKS1	M / PO	Chr17	56290344T>C	No	
MKS1	M / N	Chr17	56290344T>C	No	
TMEM67	M / N	Chr8	94772149dup	No	
TMEM67	M / N	Chr8	94772207_94772210dup	No	
TMEM67	M / N	Chr8	94777845A>T	Yes	Obese son non-carrier
TMEM67	M / N	Chr8	94777845A>T	No	
TMEM67	M / N	Chr8	94811986G>A	No	
TMEM67	M / N	Chr8	94821126T>C	No	
TMEM67	M / N	Chr8	94811986G>A	No	
TMEM67	M / N	Chr8	94811986G>A	Yes	Non-obese father carrier
TMEM67	M / N	Chr8	94792861T>C	No	
TMEM67	M / N	Chr8	94821126T>C	Yes	Obese mother non-carrier
TMEM67	M / N	Chr8	94811986G>A	No	
TMEM67	M / N	Chr8	94803512G>T	No	
TMEM67	M / N	Chr8	94811986G>A	No	
TTC8	M / N	Chr14	89310195G>A	No	

Table S5: Clinical Characteristics of the *MC4R* patients

CLINICAL CHARACTERISTICS	N
SEX	
MALE	11/18 (61%)
FEMALE	7/18 (39%)
AGE	
< 18	10/18 (56%)
≥ 18	8/18 (44%)
MODE OF INHERITANCE	
AUTOSOMAL DOMINANT	16/18 (89%)
AUTOSOMAL RECESSIVE	2/18 (11%)
ONSET OF OBESITY BEFORE AGE OF 5	14/16 (88%)
PHENOTYPE	
HYPERPHAGIA	12/16 (75%)
TYPE 2 DIABETES MELLITUS	1/16 (6.3%)
AUTISM SPECTRUM DISORDER	1/16 (6.3%)
HYPOGONADISM	1/16 (6.3%)
SPINA BIFIDA	1/16 (6.3%)
SEGREGATION ANALYSIS PERFORMED	9/18 (50%)
COSEGREGATION OF OBESITY PHENOTYPE	5/9 (56%)

Chapter 3



Genetic obesity and bariatric surgery outcome in 1014 patients with morbid obesity

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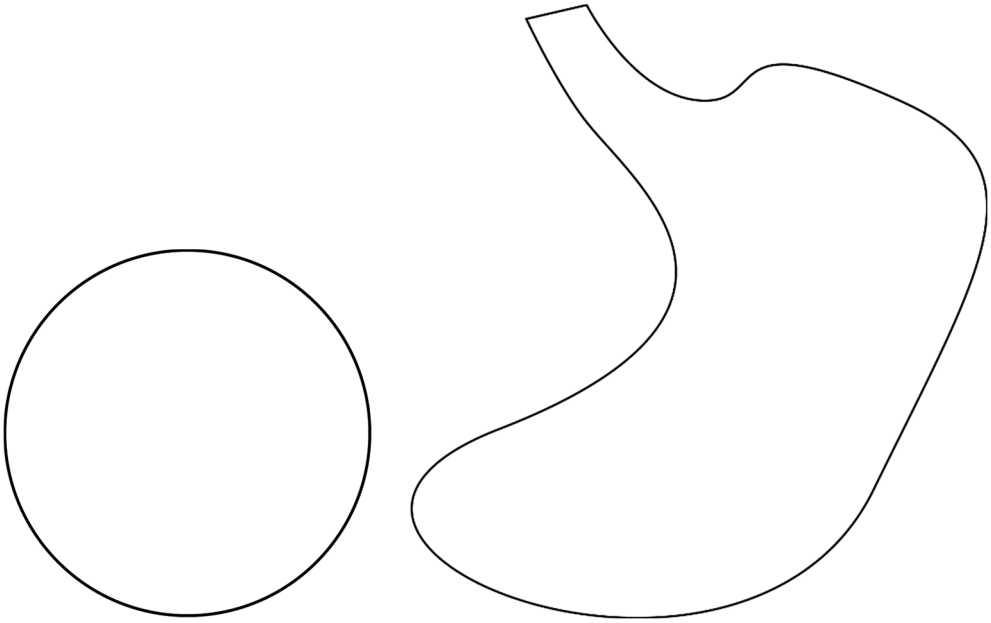

ABSTRACT

Background Mutations in the leptin-melanocortin pathway genes are known to cause monogenic obesity. The prevalence of these gene mutations and their effect on weight loss response after bariatric surgery are still largely unknown.

Objective To determine the prevalence of genetic obesity in a large bariatric cohort and evaluate their response to bariatric surgery.

Methods Mutation analysis of 52 obesity-associated genes. Patient inclusion criteria were a BMI > 50 kg/m^2, an indication for revisional surgery or an early onset of obesity (< 10 years of age).

Results A total of 1014 patients were included, of whom 30 (3%) were diagnosed with genetic obesity, caused by pathogenic heterozygous mutations in either *MC4R, POMC, PCSK1, SIM1*, or *PTEN*. The percentage total body weight loss (%TBWL) after Roux-en-Y gastric bypass (RYGB) surgery was not significantly different for patients with a mutation in *MC4R, POMC,* and *PCSK1* compared with patients lacking a molecular diagnosis. Of the confirmed genetic obesity cases, only patients with *MC4R* mutations receiving a sleeve gastrectomy (SG) showed significantly lower %TBWL compared with patients lacking a molecular diagnosis, during 2 years of follow-up.

Conclusions In this cohort of morbid obese bariatric patients, an estimated prevalence of monogenic obesity of 3% is reported. Among these patients, the clinical effects of heterozygous mutations in *POMC* and *PCSK1* do not interfere with the effectiveness of most commonly performed bariatric procedures within the first 2 years of follow-up. Patients with *MC4R* mutations achieved superior weight loss after primary RYGB compared with SG.

Introduction

The leptin-melanocortin pathway is a well-known regulatory pathway for energy balance.(1) Mutations in genes involved in this pathway are known to cause monogenic non-syndromic obesity in humans. In contrast with syndromic obesity, in which obesity is associated with congenital malformations, dysmorphic features, and/or intellectual deficit, monogenic non-syndromic obesity causes mainly obesity. Melanocortin-4 receptor (*MC4R*) gene mutations are the most common cause of monogenic non-syndromic obesity. These mutations affect proper functioning of MC4R. Melanocortin-4 receptors are located on neurons of the paraventricular nucleus of the hypothalamus.(2) Mutations in genes affecting other parts of the leptin-melanocortin pathway, such as leptin (*LEP*), leptin receptor (*LEPR*), pro-opiomelanocortin (*POMC*), and pro-protein convertase subtilisin/kexin type 1 inhibitor (*PCSK1*), also cause monogenic obesity.(3, 4, 5) Little is known about the prevalence of monogenic causes of obesity in adult populations, especially in adults undergoing bariatric surgery. Bariatric surgery is currently the most effective treatment option for obesity in adults, resulting in durable weight loss in the majority of patients.(6, 7, 8) Unfortunately, 20–30% of the patients appear to suffer from either insufficient weight loss or weight regain.(9) The amount of weight gain needed to be defined as weight regain varies widely and consensus is lacking. It has been suggested that approximately 15–25% regain of excess weight, with or without worsening of comorbidities, can be reported as weight regain.(10) The mechanisms behind weight regain are hypothesized to be either anatomical changes or inability to permanently adopt a healthy life style.(9) Another hypothesis is a possible negative effect of an underlying obesity-associated gene defect which influences the weight loss response. In the literature, data on bariatric surgery outcome in patients with genetic obesity is limited. Most studies only report (single) cases with some controversy in the weight loss outcome, varying from no different weight loss response to lower weight loss rates.(11-15) In our study, we offered genetic testing for 52 obesity- and obesity comorbidity-associated genes to selected patients undergoing bariatric surgery, with the aim to determine the weight loss response after different bariatric procedures.

Materials and Methods

Study Population

We included patients with clinically significant obesity with an age between 18 and 65 years, suitable for bariatric surgery according to the criteria of the International Federation for Surgery of Obesity and Metabolic disorders (IFSO), and offered genetic testing if they met (one of) the following inclusion criteria: BMI > 50 kg/m^2, childhood onset obesity (< 10 years of age), and/or indication for revisional surgery, either: 1. Gastric bypass procedure after failing Adjustable Gastric Banding (AGB),2. Another bariatric procedure due to insufficient weight loss or weight regain, after exclusion of anatomical problems. (Table 2)Insufficient weight loss in this study is defined as a percentage total body weight loss < 20% during

several years of follow-up (mean 70 months; range 20–182 months). Weight regain was defined as recovery of weight of > 50% of the initial amount of kilograms lost; in the majority of cases, this was combined with relapse of comorbidities. Unfortunately, in the literature, there is no consensus regarding these definitions, yet.

All patients gave written informed consent for the diagnostic test and the use of the anonymous test data. All included patients underwent bariatric surgery in a large bariatric clinic, where more than 1200 bariatric procedures are performed each year. All patients received the diagnostic test results by the doctor who ordered the test. In the event of an abnormal result, patients were referred to the clinical geneticist for genetic counseling. A brief description of molecular findings of a part of the cohort included in this study (659 patients in total) was previously described by Kleinendorst et al. 2018.(16) The bariatric surgery outcome has not been reported in this previously published article since the follow-up period was not sufficient at time of preparation of that manuscript. Therefore, the focus of this current article is mainly on the bariatric surgery outcome.

Obesity Gene Panel Analysis

The peripheral blood samples were analyzed by the ISO15189 accredited Genome Diagnostics section of the Department of Genetics, UMC Utrecht (The Netherlands). The diagnostic obesity gene panel comprises of 52 genes. Further details of these genes are provided in Supplementary Table 1. The next-generation sequencing method, bioinformatics analysis, variant filtering, and interpretation of clinical relevance were previously described by Kleinendorst et al., 2018.(16) Interpretation of clinical relevance was performed according to the ACMG standards and guidelines for the interpretation of sequence variants.(17)

Phenotyping and Segregation Analysis

Patients with abnormal results identified by the gene panel analysis were referred to a clinical geneticist. During this outpatient clinic visit, patients' medical history from birth to current status and family history (including three-generation pedigree) were recorded. Physical examination was performed to determine the presence of dysmorphic features fitting a monogenic syndromic obesity diagnosis. In some cases, segregation analysis was performed to further evaluate the impact of a genetic variant of uncertain significance (VUS).

Assessment

A standardized health program was followed by all patients before surgery, focusing on lifestyle and nutritional changes, optimizing physical activity and motivation during multiple sessions. For 5 years after bariatric surgery this program was continued with multiple sessions in the first 2 years, followed by one annual session for the next 3 years. Biochemical

analyses were performed according to the guidelines for follow-up after bariatric surgery, to check for vitamin and nutritional deficiencies, twice in the first year, followed by annual measurement. Weight loss results were determined at the same frequency.

Surgical Procedures

Primary Gastric Bypass or Sleeve Gastrectomy

Laparoscopic Roux-en-Y gastric bypass (RYGB) surgery was performed in a standardized fashion, by creating a gastric pouch with a volume of 30 to 50 mL, followed by an alimentary and biliopancreatic limb with lengths of 150 and 100 cm, respectively.(8) The sleeve gastrectomy (SG) was performed laparoscopically in a standardized fashion, performing a longitudinal resection of the greater curvature of the stomach using a 40 French catheter to ensure the correct diameter of the "sleeve stomach".(18)

Revisional Procedure

All adjustable gastric bands (AGB) were placed laparoscopically using the pars flaccid technique (SABG, Ethicon). In case of insufficient weight loss or weight regain during follow-up, removal of the band and conversion to a gastric bypass (revisional RYGB) configuration in the same session were performed, in most cases.

Statistical Analysis

Statistical analyses were performed using the Statistical Package for Social Sciences (IBMM SPSS Statistics, version 21.0.0 for Windows, IBM Corporation, Armonk, NY). The results are presented as values ± standard deviation (SD) or counts and percentages. To determine differences in demographic variables, descriptive statistics were used. Differences in continuous variables were analyzed by using the independent samples *T* test, and categorical data was analyzed by using the Fisher's exact test. To adjust for the baseline covariates, including gender, age, and preoperative BMI, a linear regression analysis was performed. All tests were two tailed and a p value < 0.05 was considered as statistically significant.

Results

Between June 2014 and September 2016, diagnostic DNA analysis of the obesity gene panel was performed in 1014 patients. Baseline characteristics for the subgroups are provided in Table 1. No significant differences in the indication for gene panel analysis between patients with a definitive molecular diagnosis compared with patients lacking a molecular diagnosis could be identified. Further details are provided in Table 2.

Table 1. Patients characteristics

Variables	Definitive diagnosis (*n* = 30)	Lacking a molecular diagnosis (*n* = 827)	*p* value
Age (years)	44.1 (± 11.7)	46.3 (± 11.3)	0.316
Number of females	21	648	0.258
BMI (kg/m^2)	48.3 (± 9.9)	45.6 (± 8)	0.088
Comorbidities:			
Diabetes mellitus	4 (13%)	149 (18%)	0.974
Insulin dependent	3 (10%)	50 (6%)	0.723
Hypertension	6 (20%)	279 (34%)	0.550
Dyslipidemia	3 (10%)	130 (16%)	0.941

All variables are expressed as mean (± standard deviation) or as number of patients (percentage), prior to surgery. *BMI*, body mass index. Diabetes mellitus, all patients had type 2 diabetes mellitus.

Table 2. Indication gene panel analysis

Inclusion criteria	Definitive diagnosis group (*n* = 30)	Lacking molecular diagnosis group (*n* = 827)	*p* value
Indication redo-surgery	6 (20%)	282 (34%)	0.675
BMI > 50 kg/m^2	11 (37%)	207 (25%)	
Early age of onset (< 10 years of age)	13 (43%)	337 (41%)	

Data are expressed as number of patients (percentage). *BMI*, body mass index. Maximum BMI in definitive diagnosis group: 70.7 kg/m^2; maximum BMI in lacking molecular diagnosis group: 91 kg/m^2

Confirmed Genetic Obesity Diagnoses

In 30 of the 1014 (3%) patients, a definitive genetic obesity diagnosis could be established. However, genealogic analysis showed that four relatives from two different families were included, so the corrected prevalence rate of monogenic obesity in this cohort is 2.8% (28/1012). None of the patients had a syndromic obesity diagnosis. Apart from one patient with a heterozygous pathogenic *PTEN* mutation, all remaining identified pathogenic gene defects affect the leptin-melanocortin pathway. Detailed mutation and patient characteristics are provided in Supplementary Table 2. Heterozygous pathogenic mutations in *MC4R* were identified in 11 patients from nine families, resulting in a prevalence of 0.9% (9/1012). A definitive genetic obesity diagnosis was established in 12 patients (1.2%) with pathogenic heterozygous mutation in *POMC*, one (0.1%) in *SIM1* and five (0.5%) in *PCSK1.* The patient in whom a known pathogenic mutation in *PTEN* was identified, diagnosis of PTEN hamartoma tumor syndrome was made. Clinical characteristics of this single case had been described elsewhere.(19)

Weight Loss After Primary RYGB

The majority of patients with a confirmed genetic obesity diagnosis due to mutations in *MC4R*, *POMC*, or *PCSK1* underwent primary RYGB surgery (57%). Percentage total body weight loss (%TBWL) compared with a control group, consisting of patients lacking a

molecular diagnosis, are provided in Fig. 1. After analyzing weight loss per gene mutation, no significant differences in %TBWL could be identified after 1 and 2 years after primary RYGB.

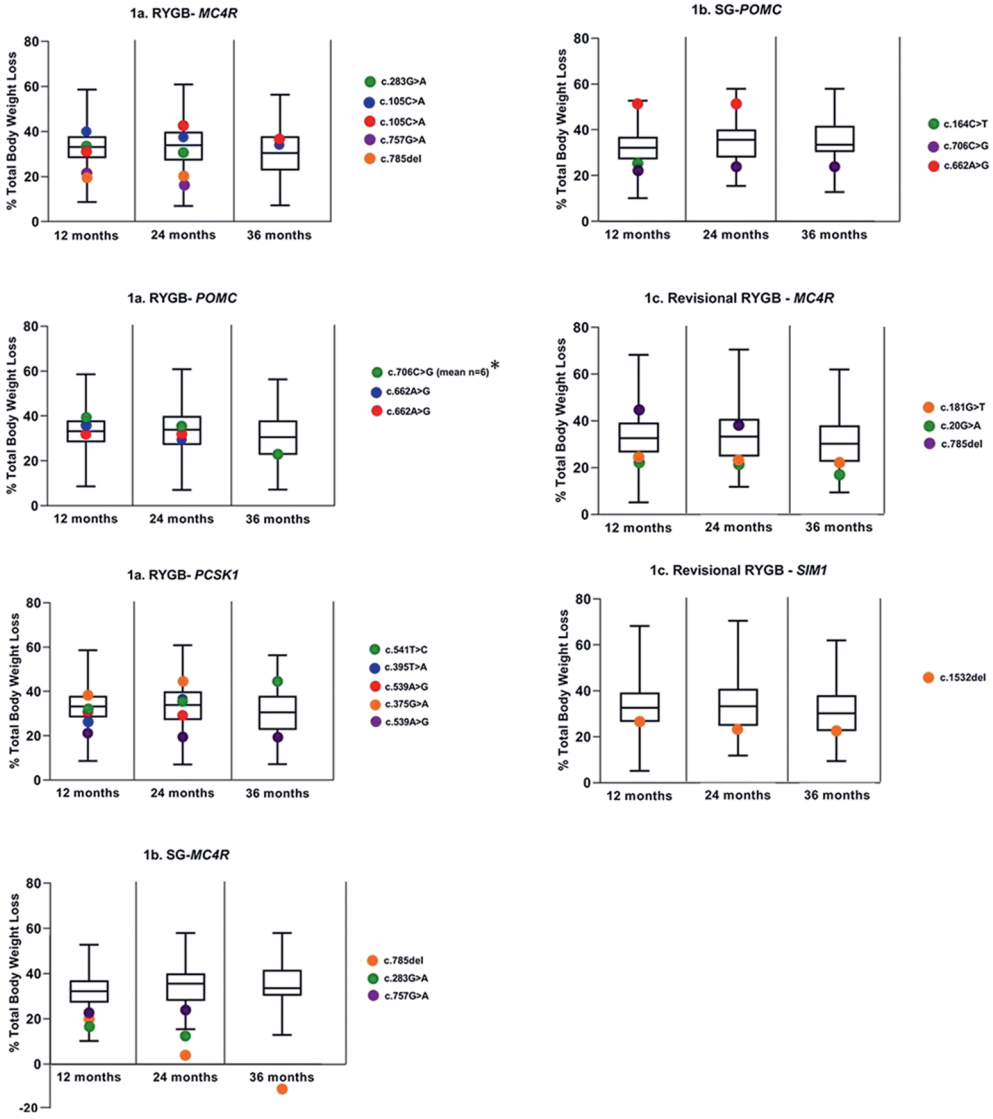

Figure 1. a–c Box plots of TBWL in patients with pathogenic variants.
Asterisk indicates mean percentage TBWL of six patients with the same mutation in POMC. The follow-up rates for the three different groups of patients lacking a molecular diagnosis are RYGB 12 months: n = 412, 24 months: n = 308, and 36 months: n = 91; SG 12 months n = 99, 24 months: n = 63 and 36 months: n = 21; Revisional RYGB 12 months: n = 248, 24 months: n = 176, and 36 months: n = 64

Weight Loss After Primary SG

Figure 1b illustrates the %TBWL compared with the patients lacking a molecular diagnosis. The three SG patients with pathogenic *MC4R* mutations achieved a %TBWL of 19.3% (± 1.3) after 12 months and 9.5% (± 3.1) after 24 months. Compared with the control group, these %TBWL were statistically significant at both time points ($p = 0.03$ and $p < 0.001$), also after adjustment for age, sex, and preoperative BMI. No significant difference in %TBWL was observed in the three patients with *POMC* mutations after SG.

Weight Loss After Revisional RYGB

Figure 1c illustrates the individual %TBWL compared with the control group of patients lacking a molecular diagnosis. Unfortunately, one patient with a *POMC* mutation was lost to follow-up within the first year after surgery. Three of the remaining patients had an *MC4R* mutation and completed 2 years of follow-up. After 12 months, %TBWL was not significantly different compared with the patients lacking a molecular diagnosis. In contrast, %TBWL at 24 months ($p = 0.009$) remains significantly different after adjustment for age, sex, and preoperative BMI ($p < 0.001$).

Possible Future Genetic Obesity Diagnoses

In an additional 34 patients (3.4%), heterozygous variants of uncertain significance (VUS) were identified: in four patients, a strong VUS was identified in *POMC* or *PCSK1* that could lead to a future genetic obesity diagnosis. In the other 30 patients, a VUS in other genes part of the leptin-melanocortin pathway was identified. For some genes, a definitive association with the human obesity phenotype has not yet been established. However, these genes are suspected to have a substantial role in the pathogenesis of obesity. Segregation analysis among family members and functional studies evaluating the effect of the specific genetic variants are necessary to assess the pathogenicity of these variants. Individual bariatric surgery outcome is provided in Supplementary Table 3.

Anamnestic Age of Onset

In 97% of the patients, the self-reported age of obesity onset was collected (Table 3). Among the patients lacking a molecular diagnosis, only 23 patients (3%) reported obesity since birth, against five patients (17%) in the group with a definitive genetic obesity diagnosis ($p < 0.001$).

Table 3. Self-reported age of obesity onset

Age of onset	Definitive diagnosis (*n* = 30)	Lacking molecular diagnosis (*n* = 827)	*p* value
From birth	5 (16.7%)	23 (2.8%)	*< 0.001*
(> 1 ≤ 10 years)	10 (33.3%)	451 (54.5%)	*< 0.001*
≥ 12 < 20 years	10 (33.3%)	153 (18.5%)	*< 0.001*
> 20 years	2 (6.7%)	98 (11.8%)	0.619
> 30 years	3 (10%)	53 (6.4%)	0.735
After pregnancy	0	23 (2.8%)	0.496
Unknown	0	26 (3.1%)	0.275

Data is expressed as number of patients (percentage). Italicized numbers indicate a significant different value

Discussion

A heterozygous obesity gene mutation was identified in 30 out of the 1014 bariatric patients (3%), resulting in a definitive genetic diagnosis. The identified pathogenic mutations are located in five out of the 52 genes that were analyzed. Except for *PTEN*, four genes are key players in the leptin-melanocortin pathway. The *MC4R* gene has been extensively studied and mutations in this gene are the most common known cause of monogenic obesity without intellectual deficit (ID).(11) The prevalence of heterozygous *MC4R* mutations was 0.9% in our bariatric cohort. In the literature, prevalence of *MC4R* mutations is dependent on the characteristics of the tested cohort with obesity and varies between 0.5 and 6%.(12, 20-23) Our reported prevalence lies in the lower quadrant of the reported prevalence in literature and can be explained by our inclusion criteria. Apart from early onset obesity, we included patients with an indication for revisional surgery and a BMI > 50 kg/m^2. As described by several authors, the prevalence of *MC4R* mutations is highest in cohorts with proven early-onset obesity. In our cohort, 45.9% of the patients reported early onset of their obesity. If we recalculate the prevalence, only including patients with early onset obesity, a prevalence of 2.5% is reached, which is more comparable with the prevalence reported in the literature.(24) In 1.2% of the tested patients, we identified heterozygous pathogenic *POMC* mutations. According to Challis et al., the prevalence of a specific heterozygous mutation in *POMC* (R236G) was 0.9% in patients with early-onset obesity compared with 0.2% in normal weight controls.(25) In our bariatric cohort, 0.8% had this specific mutation, which is in accordance with the reported prevalence in other European patient cohorts with early-onset obesity.(25)

Five patients (0.5%) had heterozygous pathogenic mutations in *PCSK1*, which is slightly lower than the reported rate of 0.83% in the literature. Four of these five heterozygous mutations were previously reported in cases that had partial PCSK1-deficiency and are associated with an 8.7-fold higher risk of becoming obese compared with lean subjects.(26, 27) One mutation (c.395T>A p.(Leu132*)) has not been previously described in literature. However, this nonsense mutation introduces a premature stop codon in exon 4 of

the *PCSK1* mRNA, which is expected to result in nonsense-mediated decay of the mRNA, thus representing a deficiency allele. Creemers et al. reported a prevalence of 0.83% of rare *PCSK1* variants among extreme obese subjects and 0.19% among obese (northern) Europeans with a BMI > 35 kg/m^2.(27) One patient (0.1%) had a heterozygous pathogenic mutation in *SIM1.* This deletion (c.1532del p.(Asn511Thrfs*58) results in a frameshift in the open reading frame of the *SIM1* mRNA, followed by a premature stop codon 58 codons downstream. This change is expected to result in the formation of a truncated protein lacking a large part of the C-terminal single-minded domain, most likely resulting in a loss-of-function. Bonnefond et al. reported four rare *SIM1* variants in seven patients out of a combined adult obese cohort of 568 patients (1.2%).(28) The prevalence of *SIM1* mutations among bariatric patients is presently unknown.

Several research groups have reported that weight loss after RYGB and AGB was not significantly different between patients with and without *MC4R* mutations, with a follow-up period up to several years.(11-15) In accordance with the literature, weight loss responses after primary RYGB in our *MC4R* patients were not significantly different to those in patients lacking a molecular diagnosis. However, this contrast with the result was seen in *MC4R* patients who underwent SG; they showed significantly different poorer weight loss compared with the patients lacking a molecular diagnosis. All three affected patients still reported persistent hunger sensations, with lower satiety than expected, which was not reported by any of the patients having primary RYGB surgery. The favorable effect of RYGB in patients with *MC4R* mutations, such as durable suppression of appetite, is known from the literature.(29) The altered bowel anatomy, created during RYGB, induces changes in levels of gut hormones (incretins), bile acids, and the microbiome. All these factors play an important role in the satiety regulating areas in the brain.(6) It is possible that these neuro-hormonal changes are more pronounced after RYGB than after restrictive procedures with an intact intestinal configuration, such as AGB and SG. Unfortunately, the relatively low number of *MC4R* patients with a SG in this study makes it difficult to draw definitive conclusions about the effect of *MC4R* mutations on weight loss in this subgroup and therefore needs to be further evaluated in larger cohorts.

To our knowledge, this is the first report on weight loss after revisional RYGB in *MC4R* and *SIM1* patients. The patients lacking a molecular diagnosis achieved comparable weight loss after revisional RYGB to that described in the literature.(30) In contrast, the *MC4R* patients in our study have insufficient weight loss after revisional RYGB at 1 and 2 years of follow-up. The patients with heterozygous mutations in *PCSK1* or *POMC* showed the same weight loss results after primary RYGB and SG as patients lacking a molecular diagnosis. Therefore, it seems that these mutations do not affect the effectiveness of primary bariatric surgery within 2 years of follow-up. However,

the number of cases is limited and long-term follow-up data are needed to determine the durability of weight loss.

The yield of the diagnostic obesity gene analysis of 3% in our bariatric cohort is relatively low, with only five out of the 52 analyzed genes harboring pathogenic mutations. In 3.4% of patients, possible pathogenic mutations (VUS) were found for which further segregation analysis or functional studies are needed to interpret the pathogenicity. Future positive segregation analyses might further increase the diagnostic yield with 3.4% to a maximum of 6.2%. In addition, the current analyses focus on known genetic causes of obesity. The gene panel was designed in 2012, and some recently identified obesity-associated genes are therefore not included in the panel. It would be of future interest to analyze these recently identified obesity-associated genes in this cohort as well. Recent large-scale genome-wide association studies (GWAS) on obesity have identified numerous common variants of small effect, whose combined effects can be captured by a genetic risk score or polygenic risk score.(31,32) Genetic risk estimated from these common variants may provide useful additional information. In combination with monogenic testing, this might help to identify individuals most at risk of morbid obesity or those that are most likely to respond to surgical treatment.

The outcomes of this study show the possible importance of screening for *MC4R* or other obesity-associated gene mutations prior to bariatric surgery. Unfortunately, it is still unknown which clinical characteristics are predictors for genetic obesity. Although previous studies suggested that higher BMIs (> 50) increases the chance of finding an underlying genetic cause, this large cohort did not support these findings.(33) Therefore, just using high BMI as a risk parameter seems inappropriate. Although *MC4R* gene analysis is currently still costly, mutation detection could be of help in determining the type of bariatric surgery and it might also be in the patients' benefit to know. In conclusion, personalized care for patients suffering from genetic obesity could eventually result in intensified follow-up programs and prevent (early) weight regain.

Conclusion

A confirmed genetic obesity diagnosis was established in 3% of our bariatric cohort. Apart from the patients with *MC4R* mutations receiving SG, weight loss was comparable between patients with or without a molecular diagnosis after 2 years of follow-up. Further long-term follow-up is necessary to determine the durability of weight loss between these two patient groups. Future research should also focus on determining the role of common variants in patients with clinically significant obesity, since the prevalence of monogenic obesity, at least caused by genes investigated here, appears to be low in this patient group.

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SUPPLEMENTARY APPENDIX

The supplementary appendix consists of three tables with additional information on the genes included in the obesity gene-panel, characteristics of patients with a pathogenic variant which confirms the obesity diagnosis, and characteristics of patients identified having variants of uncertain clinical significance (VUS).

Contents

Table S1. Obesity gene-panel and specifications

Custom Agilent SureSelect target enrichment assay followed by massive parallel sequencing on SOLiD5500XL sequencer: analysis of protein coding and flanking intronic sequences of 52 obesity and obesity comorbidity associated genes.

Gene or name of syndrome	OMIM-entry	Location (GRCh37/hg19)	Gene name	Inheritance	Symptoms, besides obesity	Name of syndrome	Association with obesity
ALMS1	606844	**2** 73612886 - 73837920	Homozygous or compound heterozygous mutations in ALMS1, centrosome and basal body associated protein (*ALSM1*)	Autosomal recessive	Retinitis pigmentosa, diabetes mellitus, hearing impairment	Alström disease	In humans
***ARL6* (also known as *BBS3*)**	608845	**3** 97483365 - 97519953	Homozygous mutations in ADP ribosylation factor like GTPase 6 (*ARL6*)	Autosomal recessive	Retinitis pigmentosa	Bardet-Biedl syndrome 3	In humans
BBS1,	209901	**11** 66278077 - 66301098	Homozygous or compound heterozygous mutations in more than 20 Bardet-Biedl associated genes	Autosomal recessive	Polydactyly, retinal defects, renal defects, hypogonadism	Bardet-Biedl syndrome	In humans
BBS2,	606151	**16** 56500748 – 56554195					
BBS4,	600374	**15** 72978527 – 73030817					
BBS5,	603650	**2** 170335688–70382432					
BBS7,	607590	**4** 122745595-					
BBS9,	607968	122791652					
		7					
	610148	33168856 - 33645680					
BBS10,		**12**					
	610683	76738254 - 76742222					
BBS12		**4** 123653857-123666098					
BDNF	113505	**11** 27676440 - 27743605	Heterozygous mutations in or chromosomal rearrangements affecting	Autosomal dominant	Hyperphagia, ADHD, memory problems, impaired pain sensation	-	In mice

			brain-derived neurotrophic factor (*BDNF*)				
CCDC28B	610162	**1** 32665987 - 32670988	Homozygous or compound heterozygous mutations in coiled-coil domain containing 28B (*CCDC28B*)	Autosomal recessive	Polydactyly, retinal defects, renal defects, hypogonadism	Bardet-Biedl syndrome 1	In humans
CEP290	610142	**12** 88442793 - 88535993	Homozygous or compound heterozygous mutations in centrosomal protein 290kda (*CEP290*)	Autosomal recessive	Retinitis pigmentosa, mental retardation and nystagmus	Bardet-Biedl syndrome 14, Joubert syndrome 5, Meckel syndrome 4	In humans
CRHR2	602034	**7** 30692200 - 30739745	Corticotropin-releasing factor receptor 2 (CRHR2)		In mice: homozygous mutations showed early termination of adrenocorticotropic hormone release	-	Pathway
FLOT1	606998	**6** 30695486 - 30710510	Flotillin 1 (*FLOT1*)		In rats: liver cells expressing human NPC1L1, require FLOT 1 and 2 for bulk endocytosis of cholesterol-enriched plasma membrane microdomains.	-	Pathway
G6PC	613742	**17** 41052814 - 41065386	Homozygous or compound heterozygous mutations in glucose-6-phosphatase, catalytic (*G6PC*)	Autosomal recessive	Manifestation during first year of life with severe hypoglycemia and hepatomegaly due to accumulation of glycogen; growth retardation, delayed puberty, hyperlipidemia.	Glycogen storage disease 1a, von Gierke disease	Obesity candidate gene
GNAS	139320	**20** 57414773- 57486247	Heterozygous mutations in GNAS complex locus (*GNAS*)	Autosomal dominant	Short stature, round face, skeletal defects, Multi-hormone resistance in	Albright hereditary osteodystrophy	In humans

					pseudohypoparathyroidism type 1A.		
IRS1	147545	**2** 227596033 - 227664475	Heterozygous mutations in insulin receptor substrate 1 (*IRS1*)	Autosomal dominant	Susceptibility to coronary artery disease, diabetes mellitus, noninsulin-dependent	-	Comorbidity gene
IRS2	600797	**13** 110406184 - 110438915	Heterozygous mutations in insulin receptor substrate 2 (*IRS2*)	Autosomal dominant	Diabetes mellitus, noninsulin-dependent	-	Comorbidity gene
IRS4	300904	**X** 107975712 - 107979651	Insulin receptor substrate 4 (*IRS4*)			-	Comorbidity gene
KIDINS220	615759	**2** 8865408 - 8977760	Heterozygous mutation in kinase D interacting substrate 220 (*KIDINS220*)	Autosomal dominant	Spastic paraplegia, ophthalmologic defects, typical facial features, macrocephaly and tall stature in first year of life.	SINO syndrome (spastic paraplegia, intellectual disability, nystagmus, obesity)	In humans
LEP	164160	**7** 127881337 - 127897681	Homozygous or compound heterozygous mutations in leptin (*LEP*)	Autosomal recessive	Hyperphagia, hypogonadotropic hypogonadism, hypothyroidism, frequent infections	-	In humans
LEPR	601007	**1** 65886248 - 66107242	Homozygous or compound heterozygous mutations in leptin receptor (*LEPR*)	Severe: autosomal recessive	Homozygous: hypogonadotropic hypogonadism, hypothyroidism, grown hormone deficiency, frequent infections	-	In humans
LZTFL1	606568	**3** 45864808 - 45957534	Homozygous or compound homozygous mutations in leucine zipper transcription factor-like 1 (*LZTFL1*)	Autosomal recessive	Polydactyly, retinal defects, renal defects, hypogonadism	Bardet-Biedl syndrome 17	In humans

MAGEL2	605283	**15** 23888691 - 23891175	Heterozygous mutations in mage family member L2 (*MAGEL2*) on the paternal allele	Autosomal dominant	Neonatal hypotonia and feeding difficulties (Parder-Willi like), behavioral abnormalities. Mild contractures to fetal akinesia	Schaaf-Yang syndrome	In humans
MC3R	155540	**20** 54823788 - 54824871	Heterozygous mutations in melanocortin 3 receptor (*MC3R*)	Autosomal dominant	Some human obese cases with heterozygous mutations and complete inactivation of the receptor.	-	Obesity candidate gene
MC4R	155541	**18** 58038564 - 58040001	Homozygous or compound heterozygous mutations in melanocortin 4 receptor (*MC4R*) Heterozygous mutations in *MC4R*	Severe: autosomal recessive Moderate: autosomal dominant	Hyperphagia, accelerated linear growth (height and occipitofrontal circumference), hyperinsulinemia	-	In humans
MCHR1	601751	**22** 41074754 - 41078818	Melanin-concentrating hormone receptor 1 (*MCHR1*)			-	Obesity candidate gene
MKKS	604896	**20** 10381657 - 10414870	Homozygous or compound heterozygous mutations in McKusick-Kaufman syndrome gene (*MKKS*), also known as *BBS6* gene	Autosomal recessive	Polydactyly, retinal defects, renal defects, hypogonadism	Bardet-Biedl syndrome 6, McKusick-Kaufman syndrome	In humans
MKRN3	603856	**15** 23810454 - 23873064	Heterozygous mutations in Makorin ring finger protein 3 (*MKRN3*)	Autosomal dominant	Precocious puberty 2	-	Obesity phenotype in precocious puberty in humans
MKS1	609883	**17** 56282803 - 56296966	Homozygous or compound heterozygous mutations in BBS13	Autosomal recessive	Polydactyly, retinal defects, renal defects, hypogonadism	Bardet-Biedl syndrome 13, Joubert	In humans

						syndrome 28, Meckel syndrome 1	
MRAP2	615410	**6** 84743475 - 84800600	Heterozygous mutations in melanocortin 2 receptor accessory protein 2 (*MRAP2*)	Autosomal dominant	Hyperphagia	-	In humans
NDN	602117	**15** 23930565 - 23932450	Necdin gene	Isolated cases	Diminished fetal activity, muscular hypotonia, mental retardation, short stature and hypogonadotropic hypogonadism.	Prader Willi syndrome	In humans
NTRK2	600456	**9** 87283466 - 87638505	Heterozygous mutations in neurotrophic receptor tyrosine kinase 2 (*NTRK2*)	Autosomal dominant	Hyperphagia, developmental delay	-	In humans
PAX6	607108	**11** 31806340 - 31839509	Heterozygous mutations in paired box homeotic gene 6 (*PAX6*)	Autosomal dominant	Aniridia, optic nerve hypoplasia, cerebral malformations	-	-
PCK1	614168	**20** 56136136 - 56141513	Homozygous mutations in phosphoenolpyruvate carboxykinase 1 (*PCK1*)	Autosomal recessive	Fasting hypoglycemia	Phosphoenol pyruvate carboxykinase deficiency, cytosolic	Pathway
PCSK1	162150	**5** 95726119 - 95769847	Homozygous or compound heterozygous mutations in proprotein convertase subtilisin/kexin type 1 (*PCSK1*) Heterozygous mutations in *PCSK1*	Severe: autosomal recessive Moderate: autosomal dominant	Neonatal diarrhea, hypothyroidism, adrenal insufficiency, diabetes insipidus	-	In humans

PHF6	300414	**X** 133507283 - 133562820	Plant homeodomain (PHd)-like finger 6 (*PHF6*)	X-linked recessive	Moderate to severe mental retardation, epilepsy, hypogonadism, hypometabolism and obesity with marked gynecomastia.	Borjeson-Forssman-Lehmann syndrome	In humans
POMC	176830	**2** 25383722 - 25391772	Homozygous or compound heterozygous mutations in proopiomelanocortin (*POMC*) Heterozygous mutations in *POMC*	Severe: autosomal recessive Moderate: autosomal dominant	Homozygous: red hair, pale skin, adrenal insufficiency Hyperphagia	-	In humans
PRKAR1A	188830	**17** 66507921 - 66547460	Heterozygous mutations in protein kinase cAMP-dependent type I regulatory subunit alpha (*PRKAR1A*)	Autosomal dominant		Acrodysostosis 1, with or without hormone resistance Carney complex, type 1 Myxoma, intracardiac Pigmented nodular adrenocortical disease	Pathway
PTEN	601728	**10** 89622870 - 89731687	Heterozygous mutations in phosphatase and tensin homologue (*PTEN*)	Autosomal dominant	Macrocephaly, autism, multiple cancers	PTEN hamartoma tumor syndrome, Lhermitte-Duclos syndrome	In humans

SIM1	603128	**6** 100832891 - 100912805	Heterozygous mutations in *SIM1* and chromosomal rearrangements affecting *SIM1*	Autosomal dominant	Autism, behavioral problems, severe obesity	-	In humans
SNRPD2	601061	**19** 46190712 - 46195827	Small nuclear ribonucleoprotein polypeptide D2 (*SNRPD2*)			-	Pathway
SNRPN	182279	**15** 25068794 - 25223870	Small nuclear ribonucleoprotein polypeptide N (*SNRPN*)	Isolated cases	Diminished fetal activity, muscular hypotonia, mental retardation, short stature and hypogonadotropic hypogonadism.	Prader Willi syndrome	In humans
SPG11	610844	**15** 44854894 - 44955876	Homozygous or compound heterozygous mutations in spatacsin vesicle trafficking associated (*SPG11*)	Autosomal recessive	Progressive spastic paraplegia, learning disabilities with or without decline, peripheral neuropathy	Spastic paraplegia 11	In humans
TBX3	601621	**12** 115108059 - 115121969	T-Box 3 (*TBX3*)	Autosomal dominant	Posterior limb deficiencies or duplications, apocrine/mammary gland hypoplasia or dysfucntion, delayed puberty in males and gentical anomalies	Ulnar-mammary syndrome	Pathway
THRB	190160	**3** 24158651 - 24536773	Heterozygous mutations in thyroid hormone receptor, beta (avian erythroblastic leukaemia viral (v-erb-a) oncogene homologue 2) (*THRB*)	Autosomal dominant	Thyroid hormone resistance	-	Comorbidity gene
TMEM67	609884	**8** 94767072 - 94831462	Homozygous or compound heterozygous mutations in transmembrane protein 67 (*TMEM67*)	Autosomal recessive	Polydactyly, retinal defects, renal defects, hypogonadism	COACH syndrome, Joubert syndrome 6, Meckel	In humans

						syndrome 3, Nephronophthisis 11, modifier of Bardet Biedl syndrome 14	
TRIM32	602290	**9** 119449581 - 119463579	Homozygous mutations in tripartite motif-containing 32 (*TRIM32*)	Autosomal recessive	Polydactyly, retinal defects, renal defects, hypogonadism	Bardet Biedl syndrome 11, Muscular dystrophy, limb girdle, autosomal recessive 8	In humans
TTC8 (also known as BBS8)	608132	**14** 89290497 - 89344335	Homozygous mutations in tetratricopeptide repeat domain 8 (*TTC8*)	Autosomal recessive	Retinitis pigmentosa 51, Polydactyly, renal defects, hypogonadism	Bardet Biedl syndrome 8	In humans
TUB	601197	**11** 8040791 - 8127659	Homozygous mutations in tubby bipartite transcription factor (*TUB*)	Autosomal recessive	Retinal dystrophy	-	In humans
WDPCP	613580	**2** 63348518 - 64054977	Homozygous or compound heterozygous mutations in WD repeat containing planar cell polarity effector (*WDPCP*)	Autosomal recessive	Congenital heart defects, hamartomas of the tongue and polysyndactyly	Bardet Biedl syndrome 15	In humans

Table S2. Pathogenic variants, confirmation of the obesity diagnosis

Pt No.	*Age (years)*	*Gender*	*Gene*	*Genotype*	*Age of onset (years)*	*Family history of obesity*	*BMI (kg/m²)*	*Inheri-tance*	*Indication genetic testing*	*Clinical features fitting diagnosis, or noteworthy*	*Country of origin*	*Bariatric procedure*
1	53	M	*MC4R*	Heterozygous: c.20G>A p.(Arg7His)	3	Father with obesity	44.7	AD	Revisional surgery	Spina bifida, amputation left leg; no counseling	The Netherlands	Revisional RYGB after AGB
2	30	M	*MC4R*	Heterozygous: c.283G>A p.(Val95Ile)	Birth	Father and brother with obesity	40.2	AD	Childhood obesity	No counseling	The Netherlands	Primary RYGB
3	64	F	*MC4R*	Heterozygous: c.105C>A p.(Tyr35*)	4	Father/sisters and son with obesity	40.8	AD	Childhood obesity	B-cell lymphoma	The Netherlands	Primary RYGB
4*	39	M	*MC4R*	Heterozygous: c.105C>A p.(Tyr35*)	Birth	Mother and sister (=patient 10), maternal aunt and her son with obesity	72.5	AD	Childhood obesity, BMI >50kg/m²	Hyperphagia	The Netherlands	Primary SG
5	38	F	*MC4R*	Heterozygous: c.482T>C p.(Met161Thr)	14	Both parents with obesity, son also obese since birth (consanguine parents)	52	AD	BMI >50kg/m²	Hyperphagia	Both parents from Afghanistan	Primary SG
6	36	F	*MC4R*	Heterozygous: c.181G>T p.(Glu61*)	8	Mother overweight, cousin with obesity since birth. Youngest son obesity since birth (consanguinity mother and father)	28.3	AD	Revisional surgery	Hyperphagia	Syria, since the age of 8 living in the Netherlands	Revisional RYGB after AGB

7**	63	M	*MC4R*	Heterozygous: c.785del p.(Phe262Serfs*4)	4	Mother, brothers and son (=patient 9) with obesity	41.6	AD	Childhood obesity	-	The Netherlands	Primary RYGB
8	41	F	*MC4R*	Heterozygous: c.785del p.(Phe262Serfs*4)	Birth	Father with obesity, and multiple family members	54.7	AD	Revisional surgery, BMI >50 kg/m^2	During childhood extreme growth in body length (no abnormalities found at that time); Hyperphagia, no satiety feeling	The Netherlands	Revisional RYGB after AGB
9**	34	M	*MC4R*	Heterozygous: c.785del p.(Phe262Serfs*4)	3	See father (=patient 7)	54.8	AD	Childhood obesity, BMI >50 kg/m^2	-	The Netherlands	Primary SG
10*	35	F	*MC4R*	Heterozygous: c.105C>A p.(Tyr35*)	4	Both families with obesity, brother (=patient 4)	43.9	AD	Revisional surgery	-	The Netherlands	Distalizatio n (after revisional RYGB after AGB)
11	44	M	*MC4R*	Heterozygous: c.757G>A p.(Val253Ile)	4	Mother with obesity	50	AD	BMI >50 kg/m^2	Gastrointestinal stromal tumor (GIST) stomach at 28th	The Netherlands	Primary RYGB
12	45	F	*PCSK1*	Heterozygous: c.375G>A p.(Met125Ile)	18	Father and eldest son with obesity	53.9	AD	BMI >50 kg/m^2	Wolff-Parkinson-White syndrome	The Netherlands	Primary RYGB

13	36	F	*PCSK1*	Heterozygous: c.539A>G p.(Asn180Ser)	Birth	Parents lean, obesity in family of father	40.6	AD	Childhood obesity	Depressions, no satiety feeling	The Netherlands	Primary RYGB
14	56	F	*PCSK1*	Heterozygous: c.395T>A p.(Leu123*)	20	No obesity in family	42.3	AD	Unknown	Cancer central nervous system	The Netherlands	Primary RYGB
15	56	F	*PCSK1*	Heterozygous: c.539A>G p.(Asn180Ser)	4	Both parents with obesity	41.7	AD	Childhood obesity	-	The Netherlands	Primary RYGB
16	37	F	*PCSK1*	Heterozygous: c.541T>C p.(Tyr181His)[#,+]	10	Mother with obesity	53.2	AD	BMI >50 kg/m^2	No counseling	The Netherlands	Primary RYGB
17	47	F	*POMC*	Heterozygous: c.706C>G p.(Arg236Gly)[+]	4	Both parents with obesity with late onset, brother with obesity	70.7	AD	Childhood obesity, BMI >50 kg/m^2	-	The Netherlands	Primary SG
18	26	M	*POMC*	Heterozygous: c.706C>G p.(Arg236Gly)[+]	Birth	Mother early onset obesity (<5 years)	57.1	AD	Childhood obesity, BMI >50 kg/m^2	Autism, hyperphagia at young childhood (parents hided all the food)	The Netherlands	Primary RYGB
19	58	F	*POMC*	Heterozygous: c.706C>G p.(Arg236Gly)[+]	4	Both parents with obesity	39.7	AD	Childhood obesity	Torsion scoliosis. Poor satiety feeling	The Netherlands	Primary RYGB

20	28	M	*POMC*	Heterozygous: c.662A>G p.(Tyr221Cys)	4	Both parents overweight, lean brother	56.5	AD	Childhood obesity, BMI >50kg/m^2	After sport trauma knee (at 8th) enormous increase in weight	The Netherlands	Primary SG
21	56	F	*POMC*	Heterozygous: c.605_616delins 18 p.(Gln202_Ser2 08delinsArgAla GlnAlaAspLeu*)	4	No obesity in family	36	AD	Childhood obesity	No counseling	The Netherlands	Revisional RYGB after AGB
22	55	F	*POMC*	Heterozygous: c.706C>G p.(Arg236Gly)$^+$	20	No obesity in family, both parents deceased at young age due to cancer. Both sisters underwent also bariatric surgery, son with obesity.	54	AD	BMI >50kg/m^2	Depressions	The Netherlands	Primary RYGB
23	62	M	*POMC*	Heterozygous: c.662A>G p.(Tyr221Cys)	8	Mother and sister obese, father very lean	38	AD	Childhood obesity	-	The Netherlands	Primary RYGB
24	42	F	*POMC*	Heterozygous: c.706C>G p.(Arg236Gly)$^+$	7	Both parents with obesity	39.5	AD	Childhood obesity	-	The Netherlands	Primary RYGB
25	31	F	*POMC*	Heterozygous: c.706C>G p.(Arg236Gly)$^+$	11	Mother with obesity, father lean	50.7	AD	BMI >50kg/m^2	Eating disorder at young age, treated	The Netherlands	Primary RYGB
26	32	F	*POMC*	Heterozygous: c.706C>G p.(Arg236Gly)$^+$	15	Mother and two brothers with obesity, father lean	48.9	AD	BMI >50kg/m^2	Uterus bicornis and one kidney; No satiety feeling	The Netherlands	Primary RYGB

27	57	F	*POMC*	Heterozygous: c.706C>G p.(Arg236Gly)[+]	12	Family members deceased at young age due to cancer. Three brothers with obesity and 1 underwent bariatric surgery	43.2	AD	Childhood obesity	Crohn's disease; MEN1 syndrome in family, for which the patient tested negative	The Netherlands	Primary SG
28	42	F	*POMC*	Heterozygous: c.662A>G p.(Tyr221Cys)[#]	18	Mother and brother with obesity	55.3	AD	BMI >50 kg/m^2	Hyperphagia	The Netherlands	Primary RYGB
29 [*]	36	F	*PTEN*	c.202T>C p.(Tyr68His)	4	Mother and sister with obesity; mother died with thyroid cancer, maternal aunt and maternal grandmother died due to breast cancer	56.7	AD	BMI >50 kg/m^2, childhood obesity	Macrocephaly	The Netherlands	Primary SG
30	49	F	*SIM1*	Heterozygous: c.1532del p.(Asn511Thrfs *58)	12	Both parents with obesity	38.9	AD	Revisional surgery	No counseling	The Netherlands	Revisional RYGB after AGB

#: these patients were identified to also have variants of uncertain clinical significance (VUS) in other genes on the gene panel, see Supplementary Table 2; ≠: this case has been described in a separate case report[22]. *: these two patients are members of family I; **: these two patients are members of family II. [+]: proven contribution to the obesity phenotype. Mutation nomenclature is according to the recommendations of the Human Genome Variation Society (HGVS). *MC4R*: NM_005912.2; *POMC*: NM_001035256.1; *PCSK1*: NM_00439.4; *PTEN*: NM_00314.4. Weight loss results after bariatric surgery of these patients are illustrated in figure 1a-c.

Table S3. Variants of uncertain clinical significance (VUS) that could possibly lead to genetic obesity diagnosis in the future
Further segregation analysis and/or functional studies will be performed to elucidate the pathogenicity of these variants.

Pt No.	*Age (years)*	*Sex*	*Gene*	*Genotype*	*Age of onset (years)*	*Family history of obesity*	*BMI (kg/m²)*	*Indication genetic testing*	*Clinical features fitting diagnosis, or noteworthy*	*Country of origin*	*Bariatric procedure*	*Weight loss (%TBWL)*
31	24	F	*POMC*	Heterozygous: c.164C>T p.(Ser55Leu)	6	Both parents with obesity	57.8	BMI <50kg/m²	No counseling	The Netherlands	Primary SG	29.6% after 1year FU
32	66	F	*PCSK1*	Heterozygous: c.328C>T p.(Arg110Cys)	10	Sister with obesity	33.4	Childhood obesity	-	The Netherlands	Revisional RYGB after AGB	21.3% after 2 years FU
33	32	F	*PCKS1*	Heterozygous: c.1991C>T p.(Ser664Phe)	8	Mother with obesity	42.4	Childhood obesity	No counseling	The Netherlands	Primary RYGB	20.2% after 1 year FU
34	48	F	*PCSK1*	Heterozygous: c.1991C>T p.(Ser664Phe)	10	Father with obesity, sister with RYGB	43.7	Childhood obesity	Type 2 diabetes mellitus	The Netherlands	Primary RYGB	27.9% after 2 years FU
35	49	F	*MC3R*	Heterozygous: c.904C>T p.(Arg302Trp)	6	Mother with obesity	44.6	Childhood obesity	Mild sleeping disorder	The Netherlands	Primary RYGB	35% after 2 years FU
36	44	M	*MC3R*	Heterozygous: c.149T>C p.(Ile50Thr)	20	Both parents and brother with extreme obesity	50.7	BMI >50kg/m²	Hyperphagia at night, sleeping disorder	The Netherlands	SADI after primary SG	15.3% after 2 years FU
37	53	F	*MC3R*	Heterozygous: c.31C>T p.(Gln11*)	12	Mother with obesity	40	Revisional surgery	Addison's disease, hyperphagia, sleeping disorder	The Netherlands	Revisional RYGB after AGB	0% after 2 years FU
38	42	F	*MC3R*	Heterozygous: c.431T>C p.(Val144Ala)	Birth	Father with RYGB	40.3	Childhood obesity	No counseling	The Netherlands	Primary RYGB	25.6% after 1 year FU
39	56	F	*MC3R*	Heterozygous: c.446C>T p.(Ala149Val)	10	Brother and niece with RYGB	41.7	Childhood obesity	-	The Netherlands	Primary RYGB	20% after 2 years FU

40	53	F	*SIM1*	Heterozygous: c.704G>A p.(Arg235His)	4	No obesity in family	55.3	BMI >50kg/m^2	No counseling, type 2 diabetes mellitus	The Netherlands	Primary RYGB	35.6% after 1 year FU
41	51	F	*SIM1*	Heterozygous: c.457+9G>A p.?	30	Mother and brother with obesity	56.3	BMI>50kg/ m^2	-	Parents from Aruba and Curacao	Primary RYGB	26% after 2 years FU
42	42	F	*SIM1*	Heterozygous: c.742C>G p.(Leu242Val)	3	Mother with obesity	40.3	Childhood obesity	-	The Netherlands	Primary RYGB	40.9% after 1 year FU
43	48	F	*SIM1*	Heterozygous: c.1649G>A p.(Arg550His)	3	Mother with obesity	49.9	Childhood obesity	Attention deficit hyperactivity disorder,depression	The Netherlands	Primary RYGB	15.1% after 2 years FU
44	56	M	*SIM1*	Heterozygous: c.2144G>T p.(Gly715Val)	8	No obesity in family	36.9	Childhood obesity	Dyslipidemia	The Netherlands	Primary RYGB	28.8% after 2 years FU
45	43	M	*BDNF*	Heterozygous: c.440G>A p.(Trp147*)	5	Mother with obesity	43.6	Childhood obesity	-	The Netherlands	Primary RYGB	18% after 2 years FU
46	53	F	*BDNF*	Heterozygous: c.133A>C p.(Ser45Arg)	10	Sister with obesity	40.1	Childhood obesity	-	The Netherlands	Primary RYGB	21% after 1 year FU
47	50	F	*LEPR*	Heterozygous: c.1717C>A p.(Arg573Ser)	Birth	Mother of patient no. 48	40.5	Childhood obesity	-	The Netherlands	Primary RYGB	7% after 2 years FU
48	27	F	*LEPR*	Heterozygous: c.1717C>A p.(Arg573Ser)	3	Daughter of patient no. 47	43.2	Childhood obesity	-	The Netherlands	Primary RYGB	27.3% after 1 year FU
49	64	F	*LEPR*	Heterozygous: c.1835G>A p.(Arg612His)	3	Mothers family with obesity	38.9	Revisional surgery	Diabetes insipidus	The Netherlands	Revisional RYGB after AGB	34.2% after 2 years FU
50	47	F	*LEPR*	Heterozygous: c.2861G>T p.(Cys954Phe)	16	Mother with obesity	54.6	BMI >50kg/m^2	-	The Netherlands	Primary RYGB	36.1% after 1 year FU
51	37	F	*LEPR*	Heterozygous:	2	Both parents with obesity	41.7	Childhood obesity	Immature teratoma	The Netherlands	Primary RYGB	28% after 1 year FU

				c.3414dup p.(Ala1139Cysfs *16)								
52	23	F	*MRAP2*	Heterozygous: c.373C>T p.(Arg125Cys)	2	Both parents with obesity	44.9	Childhood obesity	-	The Netherlands	Primary SG	42.1% after 2 years FU
53	54	F	*MRAP2*	Heterozygous: c.373C>T p.(Arg125Cys)	3	No obesity in family	63.3	BMI >50kg/m^2	-	The Netherlands	Revisional RYGB after AGB	15.8% after 1 year FU
54	49	F	*MRAP2*	Heterozygous: c.373C>T p.(Arg125Cys)	3	Father with obesity	49.7	Childhood obesity	-	The Netherlands	Revisional RYGB after AGB	26.3% after 1 year FU
55	33	F	*MRAP2*	Heterozygous: c.373C>T p.(Arg125Cys)	8	Both parents with obesity	53.9	BMI >50kg/m^2	-	The Netherlands	Primary RYGB	36% after 1 year FU
56	41	F	*MRAP2*	Heterozygous: c.373C>T p.(Arg125Cys)	4	Mother with RYGB	37.7	Childhood obesity	No counseling	The Netherlands	Revisional RYGB after AGB	17.7% after 1year FU
57	51	F	*MRAP2*	Heterozygous: c.373C>T p.(Arg125Cys)	3	Mother with obesity	47.1	Childhood obesity	No counseling	The Netherlands	Primary RYGB	31.7% after 2 years FU
58	68	M	*MCHR1*	Heterozygous: c.950G>A p.(Arg317Gln)	3	Both parents with obesity	42.3	Childhood obesity	Insulin dependent diabetes mellitus	The Netherlands	Primary RYGB	25.2% after 2 years FU
59	45	F	*MCHR1*	Heterozygous: c.950G>A p.(Arg317Gln)	4	Mother with obesity	39.3	Childhood obesity	No counseling	The Netherlands	Primary RYGB	32.7% after 2 years FU
60	25	F	*MCHR1*	Heterozygous: c.694G>C p.(Val232Leu)	1	Both parents with obesity	52	BMI >50kg/m^2	-	The Netherlands	Primary SG	20.1% after 2 years FU
61	37	F	*MCHR1*	Heterozygous: c.950G>A p.(Arg317Gln)	9	Father with obesity	46.1	Childhood obesity	-	The Netherlands	Primary RYGB	35.8% after 1 year FU
15$^{\$}$	37	F	*MCHR1*	Heterozygous: c.417G>C p.(Lys139Asn)	10	Mother with obesity	53.2	BMI >50kg/m^2	No counseling	The Netherlands	Primary RYGB	37.8% after 2 years FU

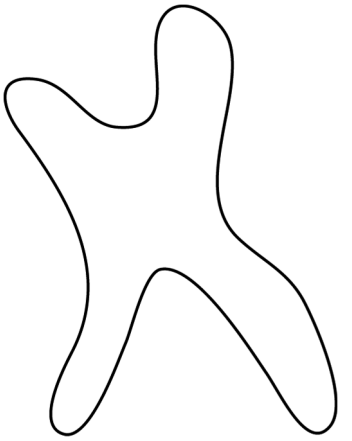
27^	42	F	*MCHR1*	Heterozygous: c.950G>A p.(Arg317Gln)	18	Mother and brother with obesity	55.3	BMI >50kg/m^2	Hyperphagia	The Netherlands	Primary RYGB	28.1% after 2 years FU
62	28	F	*MCHR1*	Heterozygous: c.950G>A p.(Arg317Gln)	2	Mother with RYGB	37	Childhood obesity	-	The Netherlands	Primary SG	16.9% after 2 years FU
63	37	F	*MCHR1*	Heterozygous: c.417G>C p.(Lys139Asn)	3	Mother with obesity	53.2	BMI >50kg/m^2	No counseling	The Netherlands	Primary RYGB	37.8% after 2 years FU
64	45	F	*MCHR1*	Heterozygous: c.950G>A p.(Arg317Gln)	Birth	Mother with obesity	39.3	Childhood obesity	No counseling	The Netherlands	Primary RYGB	32.7% after 2 years FU

%TBWL: percentage total body weight loss; FU: follow-up; Childhood obesity was stated as the anamnestic age of obesity onset; No counseling: these patients did not visit the outpatient clinic for further phenotyping. -; no noteworthy features. $^{\$}$See table 4, this patient also has a heterozygous pathogenic mutation in *PCSK1*; ^ see table 4, this patient also has a heterozygous pathogenic mutation in *POMC*. Mutation nomenclature is according to the recommendations of the Human Genome Variation Society (HGVS). *POMC*: NM_ 001035256.1; *PCSK1*: NM_00439.4; *MC3R:* NM_ 019888.3; *SIM1:* NM_005068.2; *BDNF*: NM_170735.5; *LEPR:* NM_002303.5; *MRAP2:* NM_138409.2; *MCHR1:* NM_00529

Part II

EXTENSIVE PHENOTYPING TO DISTINGUISH RARE GENETIC OBESITY DISORDERS FROM COMMON OBESITY

Chapter 4



Identifying underlying medical causes of pediatric obesity: Results of a systematic diagnostic approach in a pediatric obesity center

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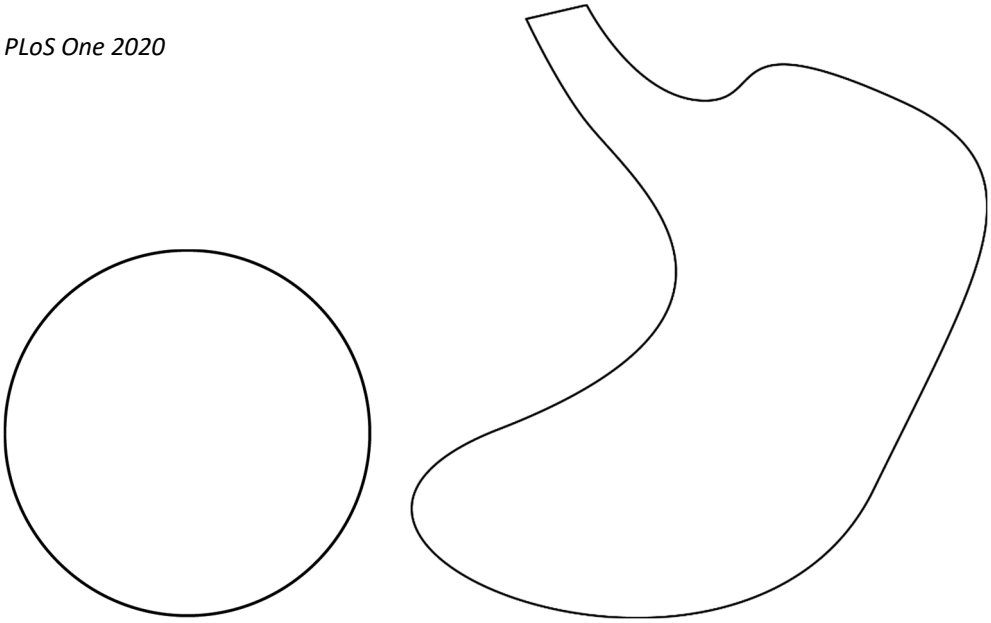

ABSTRACT

Background Underlying medical causes of obesity (endocrine disorders, genetic obesity disorders, cerebral or medication-induced obesities) are thought to be rare. Even in specialized pediatric endocrinology clinics, low diagnostic yield is reported, but evidence is limited. Identifying these causes is vital for patient-tailored treatment.siz

Objectives To present the results of a systematic diagnostic workup in children and adolescents referred to a specialized pediatric obesity center.

Methods This is a prospective observational study. Prevalence of underlying medical causes was determined after a multidisciplinary, systematic diagnostic workup including growth charts analysis, extensive biochemical and hormonal assessment and genetic testing in all patients.

Results The diagnostic workup was completed in n = 282 patients. Median age was 10.8 years (IQR7.7–14.1); median BMI +3.7SDS (IQR +3.3-+4.3). In 54 (19%) patients, a singular underlying medical cause was identified: in 37 patients genetic obesity, in 8 patients cerebral and in 9 patients medication-induced obesities. In total, thirteen different genetic obesity disorders were diagnosed. Obesity onset <5 years ($p = 0.04$) and hyperphagia ($p = 0.001$) were indicators of underlying genetic causes, but only in patients without intellectual disability (ID). Patients with genetic obesity with ID more often had a history of neonatal feeding problems ($p = 0.003$) and short stature ($p = 0.005$). BMI-SDS was not higher in patients with genetic obesity disorders ($p = 0.52$). Patients with cerebral and medication-induced obesities had lower height-SDS than the rest of the cohort.

Conclusions To our knowledge, this is the first study to report the results of a systematic diagnostic workup aimed at identifying endocrine, genetic, cerebral or medication-induced causes of pediatric obesity. We found that a variety of singular underlying causes were identified in 19% of the patients with severe childhood obesity. Because of this heterogeneity, an extensive diagnostic approach is needed to establish the underlying medical causes and to facilitate disease-specific, patient-tailored treatment.

Introduction

Obesity is a multifactorial disease that has become one of the greatest health challenges of our time.(1) The prevalence of severe obesity in children and adolescents (as defined by the World Health Organization and the International Obesity Task Force (IOTF) was recently shown to range from 1.7% to 6.3% in several countries.(2-4) Body mass index is strongly influenced by genetic susceptibility with an estimated heritability of 40–70%.(5, 6) Most children and adolescents with obesity do not have singular underlying medical disorders causing their obesity, such as endocrine disorders, genetic obesity disorders, cerebral or medication-related causes.(7) The pathophysiologic mechanisms of the underlying medical conditions causing obesity are widely varied, leading to the suggestion to talk about "different diseases causing obesity" or "obesities".(8) Establishing an underlying diagnosis can give insight into the clinical course of the obesity, and lead to tailored monitoring and treatment.(9) In addition, it ends the diagnostic odyssey and can reduce the stigma that patients are confronted with.(10, 11) Since pharmacological treatment for patients with genetic defects affecting the leptin-melanocortin pathway (the hypothalamic system that controls appetite and energy expenditure) is currently being evaluated in clinical trials, identifying these diseases becomes even more relevant.(8, 11, 12)

It is difficult to assess which patients should be evaluated for underlying causes. The current international clinical practice guideline for the evaluation and treatment of pediatric patients with obesity was published in 2017 by the Endocrine Society (ES).(13) In this guideline, clinicians are guided through the diagnostic process. After medical history-taking and physical examination, specific additional diagnostic steps are suggested depending on the findings. In short, endocrine evaluation is recommended in patients with reduced growth velocity; evaluation of hypothalamic obesity in patients with central nervous system (CNS) injury, and re-evaluation of drug choice in patients using antipsychotic drugs. In selected cases, genetic testing is recommended, e.g., in patients displaying extreme early-onset obesity (<5 years) and severe hyperphagia, which are considered cardinal features of genetic obesity disorders. The genetic tests mentioned in the guideline range from karyotyping to DNA diagnostics for deficiencies in the leptin-melanocortin pathway.

As of yet, studies that systematically screen for the underlying medical causes mentioned in the ES guideline in children and adolescents with obesity have not been performed. Previous studies on genetic obesity disorders report an underlying causative genetic defect in 2–5% of non-consanguineous pediatric patients with severe obesity, but prevalence of the other underlying medical causes of obesity has not been studied. (13–15) Therefore, our primary aim was to analyze the results of a thorough diagnostic workup in a cohort of patients who had been referred to the pediatric division of a specialized tertiary obesity center. Our diagnostic approach included broad evaluation for each patient of all possible underlying medical causes of obesity as mentioned in the ES guideline: endocrine and

genetic disorders, as well as cerebral injury and medication use. Moreover, we compared the detailed clinical phenotype of these patients to evaluate whether the patients with underlying medical causes of obesity can be distinguished from those without an underlying medical cause.

Methods

For this analysis, medical data of children and adolescents aged 0–18 years visiting Obesity Center CGG (Dutch: *Centrum Gezond Gewicht*; English: *Centre for Healthy Weight*) were analyzed. Obesity Center CGG is a Dutch multidisciplinary referral center for obesity consisting of a collaboration between the departments of Pediatrics, Internal Medicine and Surgery of the academic hospital Erasmus MC and collaborating general hospitals Maasstad Ziekenhuis and Franciscus Gasthuis.

In this prospective, observational study, informed consent was obtained at the initial visit according to Dutch law: written informed consent was obtained from parents and children >12 years; for children below age 12 years oral assent was additionally obtained. This also included separate consent forms for genetic testing. The study was approved by the medical ethics committee of the Erasmus MC (MEC-2012-257). Pediatric patients were referred to Obesity Center CGG for diagnostic evaluation (due to suspicion of underlying causes of obesity, severe obesity, or resistance to combined lifestyle intervention), personalized therapeutic advice, or participation in a combined lifestyle intervention (Fig 1).(16) All consecutive patients who provided written informed consent were included at the university medical center Erasmus MC-Sophia Children's Hospital from 2015 to August 2018. From 2016 to August 2018, the collaborating general hospital Maasstad Ziekenhuis also included patients with a suspicion of an underlying medical cause of obesity. Exclusion criteria for this study were inability or refusal to give informed consent, refusal to undergo genetic testing, or not completing the standardized diagnostic approach (Fig 1). A standardized diagnostic approach was applied for all patients (Fig 2), discussed below and in more detail in the S1 Appendix, aimed at identifying underlying endocrine, genetic, cerebral, and medication-induced main causes of obesity. At study entry, medical history-taking, physical examination and extensive assessment of growth charts were performed by a pediatric endocrinologist or pediatrician supervised by a pediatric endocrinologist.

A few weeks after the initial visit, patients returned to the outpatient clinic where blood was drawn after an overnight fast for biochemical and hormonal evaluation, and genetic diagnostics. All patients and/or their parents were asked to fill out several questionnaires regarding physical activity, eating behavior, sleeping behavior, stress, and quality of life. Furthermore, all patient records were screened by a clinical geneticist. In case of high suspicion of genetic obesity or abnormal genetic test results, patients were seen by a clinical geneticist at the outpatient clinic. Patients who visited the academic center were also seen

by a pediatric physiotherapist, pedagogist, and pediatric dietician. Additional diagnostics (i.e., further genetic testing, neuropsychological or radiologic assessments) were performed when clinically indicated following international clinical guidelines. After the diagnostic procedure, it was assessed for each patient whether an endocrine, genetic, cerebral or medication-induced main underlying cause of obesity could be diagnosed. Contributing factors to weight gain (e.g. sleep deprivation, screen time) were not considered as main underlying causes of obesity. After the diagnostic workup, a patient-tailored treatment plan was designed by the multidisciplinary team in which all relevant findings were incorporated, including advice regarding diet and physical activity, medical treatment (regarding comorbidities) or referral to combined lifestyle intervention, parent support center, psychologist, or psychiatrist. This personalized treatment plan was discussed with the patient and parents and tailored to their personal situation and needs.

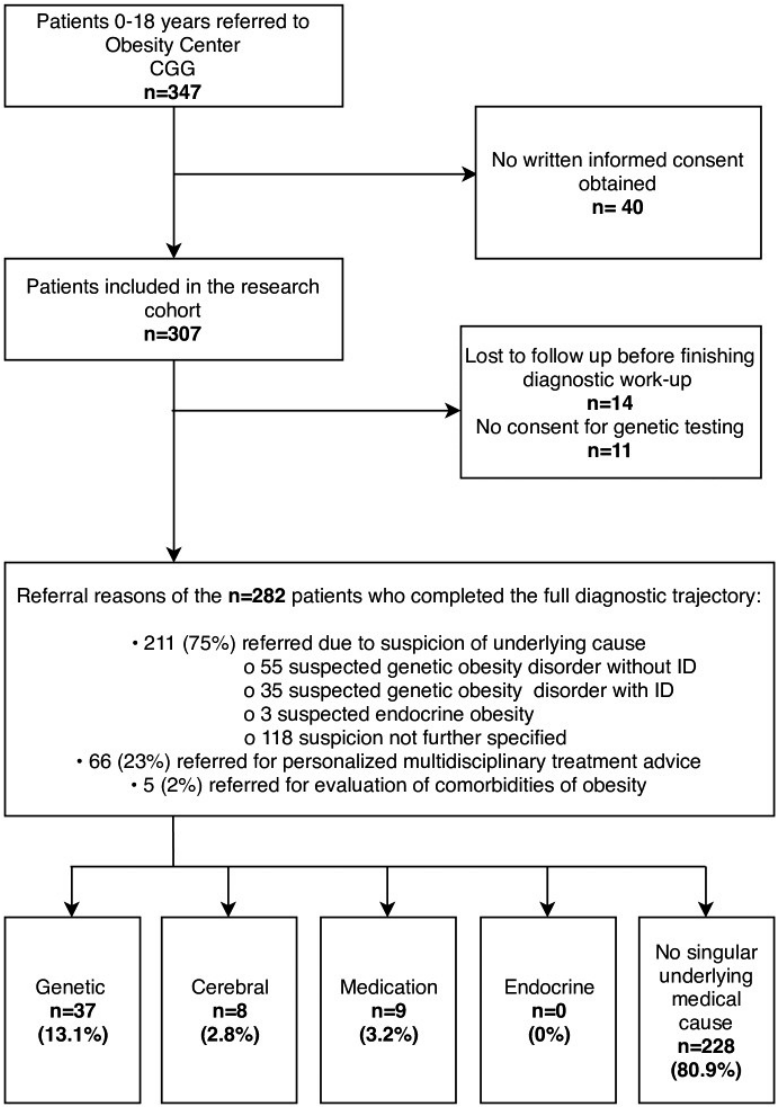

Figure 1. Study flow chart

Flow chart indicating the inclusion of participants and diagnoses established in our cohort. Abbreviations: CGG, Dutch: *Centrum Gezond Gewicht*; English: *Centre for Healthy Weight*; ID, intellectual disability.

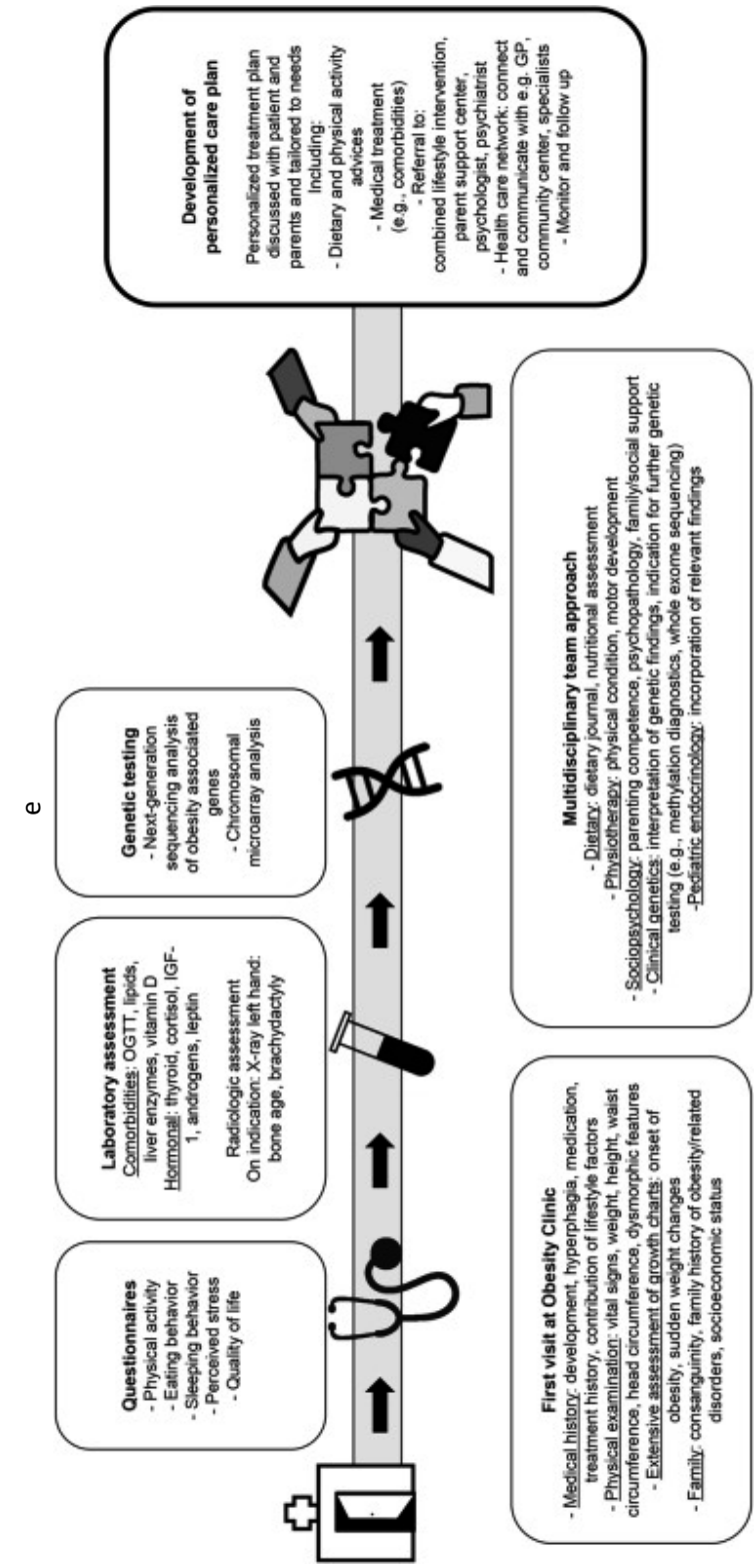

Figure 2. Diagnostic approach

Systematic diagnostic approach for children and adolescents with obesity and a suspicion of an underlying medical cause. Abbreviations: OGTT, oral glucose tolerance test; IGF-1, Insulin-like growth factor 1; GP, general practitioner.

Assessments

The features that were assessed during the diagnostic workup are summarized below (details in the S1 Appendix).

Phenotypic features

Clinical history-taking and physical examinations were performed following the Dutch pediatric obesity guideline, including evaluation of neonatal feeding, weight-inducing medication use, development, dysmorphic features, or congenital anomalies.(17) Height, weight and head circumference were measured rounded to the nearest decimal. The Dutch national growth charts, which use the definition of pediatric obesity by Cole *et al*., were used to calculate standard deviation scores (SDS).(3, 18) Severe obesity was defined by the IOTF definition as a BMI ≥ the age- and sex-specific IOTF BMI-values corresponding to a BMI of 35 kg/m^2 at age 18 years.(3) Each patient's growth charts were studied in detail to determine the age of onset of obesity and to evaluate the presence of sudden weight changes. If sudden weight changes were present, it was determined whether these changes were associated with cerebral injury (e.g., tumor in the hypothalamic region) or use of known weight-inducing medication. Short stature was defined as a height-for-age z-score <2 SDS or height-for-age <-1.6 SDS compared to target height; tall stature as a height-for-age z-score >2 SDS or height-for-age >2 SDS compared to target height.(19, 20)

Intellectual disability was determined by the DSM-5 (Diagnostic and Statistical Manual of Mental Disorders 5) definition of intellectual disability or an IQ score ≤70. Family histories of bariatric surgery and extreme obesity (BMI > 40 kg/m^2 for adults, or corresponding pediatric value) were obtained for the past three generations.(3) Information on consanguinity was obtained from questionnaires and additionally from the regions of homozygosity identified by SNP microarray analysis (see below). Presence of hyperphagia was determined by the physician, based on the child's or parents' answers regarding hunger, e.g., satiation and satiety, preoccupation with food, night eating, secret eating, food-seeking behavior, and the distress that accompanies the child's hunger or obsession with food.(21) Patients were considered Dutch if patient and both parents were born in The Netherlands; otherwise, patients were classified as having a migration background.(22) Presence of psychosocial/psychiatric problems was defined as the presence of an established DSM-5 diagnosis (with the exception of intellectual disability) or social problems for which official authorities were involved, such as child protective services. Additionally, Dutch neighborhood socioeconomic status z-scores were calculated. These summarize average income, education and unemployment in postal code areas to provide an estimate of the socioeconomic status of patients.(23) Finally, the contribution of lifestyle factors was assessed. As lifestyle factors play a role in every case of obesity, the multidisciplinary team determined if lifestyle factors were the most important contributor to the obesity for each patient without an underlying medical diagnosis. For example, this label determination was

used for patients without an underlying medical diagnosis who reported that obesity started during the divorce of their parents and consequently never resolved. This was subsequently objectified in their growth charts.

Laboratory assessment

Laboratory assessment was performed for all patients. These consisted of screening for comorbidities of obesity, including standard oral glucose tolerance test, lipids, liver enzymes, vitamin D status and hormonal assessment, i.e., thyroid hormones, cortisol, insulin-like growth factor 1, androgens, and leptin. Further details are provided in the S1 Appendix.

Genetic testing

Obesity gene panel sequencing and single nucleotide polymorphism (SNP) microarray analysis were performed in a diagnostic setting for all patients. Three diagnostic obesity gene panel tests successively became available in The Netherlands during the time span of the study (S1 Appendix). All patients were tested at least for the most important genetic obesity disorders mentioned in the ES guideline, such as *GNAS*, *LEP*, *LEPR*, *MC4R*, *PCSK1*, *POMC*, and *SIM1*.(13) Details and complete gene lists are provided in the S1 Appendix. Obesity gene panel sequencing was performed in the ISO 15189 accredited genetic diagnostics laboratories of Amsterdam UMC and UMC Utrecht. Chromosomal microarray analysis and additional diagnostic tests were also performed at the ISO 15189 genetic diagnostics laboratories of other Dutch academic centers. Identified variants were compared with in-house and public databases to exclude common variants. Variants were classified according to the American College of Medical Genetics and Genomics (ACMG) guideline.(24) Family segregation studies were performed if necessary to clarify the pathogenicity of a variant of uncertain significance (VUS) or copy number variation (CNV). Interpretation of found variants was performed in a diagnostic setting according to the ACMG guideline. Variants of uncertain significance were not classified as genetic obesity disorder, but as a VUS/CNV that possibly explains the obesity phenotype, for which functional studies or other evidence for pathogenicity are necessary. All patients were evaluated by a clinical geneticist specialized in genetic obesity disorders to see whether further genetic testing (e.g., Prader-Willi syndrome (PWS) and Temple syndrome diagnostics, whole exome sequencing) was warranted, for example in case of unexplained intellectual disability, short stature, neonatal hypotonia, multiple congenital anomalies or other signs and symptoms of genetic obesity disorders as mentioned in the ES guideline.(13)

Definition of underlying medical causes of obesity
We used the following definitions of main underlying medical causes of obesity:

Genetic obesity was diagnosed when genotyping revealed known pathogenic variants in obesity-associated genes which matched the clinical phenotype. Likely pathogenic variants, as defined by the American College of Medical Genetics and Genomic (ACMG) guideline were only considered as causative if the clinical phenotype of the patient matched with the found genotype (according to the clinical features mentioned in the ES guideline) and segregation analysis was indicative as well.(24, 13) For genetic obesity disorders not mentioned in the ES guideline, the typical phenotype was based on literature review.(25–32)

Endocrine obesity: Cushing's syndrome and clinical hypothyroidism were considered endocrine causes of obesity. Additional diagnostics for Cushing's syndrome were performed in the presence of impaired growth velocity coinciding with sudden weight gain, Cushingoid phenotype features, and abnormal laboratory results.(13, 33)

Cerebral injury was diagnosed as the cause of obesity in the presence of CNS injury affecting the hypothalamic centers for weight regulation due to craniopharyngioma surgery, meningitis or ischemic damage, coinciding with a sudden progression of obesity (seen as a clear visual slope discontinuity in the growth curve from the time of CNS injury onwards) and the absence of other plausible explanations for the sudden weight gain.

Medication-induced obesity was diagnosed in the presence of start or intensification of known weight-inducing medication (i.e., corticosteroids, anti-epileptic, anti-depressant and anti-psychotic drugs) coinciding with a sudden progression of obesity (seen as a clear visual slope discontinuity in the growth curve) and the absence of other plausible explanations for the sudden weight gain.(34-38)

Analysis
Statistical analysis was performed using SPSS version 24.0 [IBM Corp. Armonk, NY]. Data are presented as median (interquartile range; IQR) and maximum, or mean (standard deviation; SD) and maximum, as appropriate. Differences in features between patients with genetic obesity disorders and patients without a singular underlying medical cause of obesity were analyzed using the chi-squared test, Fisher's exact test, independent sample t-test or Mann-Whitney U test, as appropriate. Two-sided p-values <0.05 were considered statistically significant, as we interpreted these comparisons as hypothesis-generating. For the same reason, we decided not to perform formal statistical testing for comparisons between other patient subgroups due to the small subgroup sizes.

Results

Patient characteristics

In total, 347 patients were referred to Obesity Center CGG during the time span of this study (Fig 1). Of these patients, 282 patients underwent the complete diagnostic workup and were included in these analyses. The majority of these patients presented at the academic hospital (222; 78.7%). Most patients were referred because of suspicion of an underlying cause (Fig 1). All 282 patients underwent the described gene panel analysis and chromosomal microarray analysis. After consulting with a clinical geneticist, additional genetic diagnostics were performed for 77 patients. The most important modalities were PWS diagnostics in 31 patients; whole exome sequencing in 27 patients; maternal UPD14 diagnostics in 21 patients. Median BMI for age was +3.7 SDS (IQR +3.3-+4.3), indicating severe obesity (Table 1). Most patients were Dutch (183/282, 64.9%); 99/282 (35.1%) had a migration background. In 67/282 (23.8%) of the patients intellectual disability (ID) was present.

Table 1. Group characteristics of the study population

		All patients	Genetic obesity disorders			Cerebral obesity	Medication-induced obesity	No definite singular underlying medical diagnosis
		Total group	Genetic obesity disorders without ID	Genetic obesity disorders with ID	Total group	Total group	Total group	Total group
		n = 282	n = 19	n = 18	n = 37	n = 8	n = 9	n = 228
Patient characteristics								
Age at initial visit	Median (IQR) [max]	10.8 (7.7–14.1) [18.0]	10.0 (2.9–14.6) [17.7]	11.2 (7.1–14.7) [16.3]	10.0 (6.0–14.6) [17.7]	11.9 (10.3–16.6) [17.5]	12.3 (9.1–14.8) [17.3]	10.7 (7.7–13.6) [18.0]
Female	n (%)	165 (59%)	14/19 (74%)	12/18 (67%)	26/37 (70%)	5/8 (63%)	5/9 (56%)	129/228 (57%)
Early-onset <5 years	n (%)	182 (65%)	18/19[‡] (95%)	12/18 (67%)	30/37[†] (81%)	4/8 (50%)	4/9 (44%)	146/228 (64%)
Hyperphagia	n (%)	113 (40%)	15/19[‡] (79%)	9/18 (50%)	24/37[‡] (65%)	2/8 (25%)	3/9 (33%)	84/228 (37%)
Anthropometric features								
Height SDS	Mean (SD) [max]	+0.5 (1.3) [+4.2]	+1.1 (1.4) [+4.2]	-0.4[‡] (1.3) [+1.5]	+0.3 (1.5) [+4.2]	-0.3 (0.5) [+0.3]	-0.3 (0.7) [+1.5]	+0.6 (1.3) [+3.7]
Weight SDS	Mean (SD) [max]	+3.7 (1.2) [+7.1]	+4.6[†] (1.5) [+7.0]	+2.3[‡] (1.5) [+5.2]	+3.5 (1.9) [+7.0]	+3.4 (1.0) [+4.7]	+3.4 (0.5) [+4.1]	+3.8 (1.1) [+7.1]
BMI SDS	Median (IQR) [max]	+3.7 (+3.3 - +4.3) [+8.9]	+4.2 (+3.5 - +4.7) [+8.9]	+3.1[‡] (+2.4 - +3.5) [+5.5]	+3.5 (+2.8 - +4.4) [+8.9]	+3.4 (+3.2 - +4.2) [+5.5]	+3.7 (+3.4 - +4.0) [+4.2]	+3.8 (+3.3 - +4.3) [+6.6]
Other clinical features								
Head circumference SDS	Mean (SD) [max]	+1.4 (1.2) [+4.9]	+2.0 (1.2) [+3.9]	+0.9 (1.5) [+3.8]	+1.4 (1.5) [+3.9]	+0.8 (1.0) [+2.1]	+0.2 (1.0) [+0.8]	+1.4 (1.1) [+4.9]
History of neonatal	n (%)	17 (6%)	0/19	5/18[‡] (28%)	5/37 (14%)	1/8 (13%)	0/9	11/228 (5%)

feeding problems								
Autism	n (%)	37 (13%)	1/19 (5%)	2/18 (11%)	3/37 (8%)	0/8	2/9 (22%)	32/228 (14%)
Parents with obesity	n (%)	190 (67%) of which 68 both	10/19 (53%) of which 1 both	9/18 (50%)	19/37[†] (51%) of which 1 both	3/8 (38%)	7/9 (77%) of which 1 both	161/228 (70%) of which 66 both
Parents with history of bariatric surgery	n (%)	34 (12%) of which 3 both	1/19 (5%) 1 M	1/18 (6%) 1 M	2/37 (5%)	0/8	2/9 (22%)	30/228 (13%) of which 3 both
Consanguinity	n (%)	24 (9%)	2/19 (11%)	0/18	2/37 (5%)	1/8 (13%)	1/9 (11%)	20/228 (9%)
Psychosocial problems	n (%)	130 (46%)	3/19[‡] (16%)	4/18[†] (22%)	7/37[‡] (19%)	3/8 (38%)	5/9 (56%)	115/228 (50%)
Current/past use of weight-inducing medication	n (%)	78 (28%)	5/19 (26%)	2/18 (11%)	7/37 (19%)	3/8 (38%)	9/9 (100%)	59/228 (26%)
Evidently dysmorphic appearance and/or congenital anomaly	n (%)	49 (17%)	1/19 (5%)	12/18[‡] (67%)	13/37[‡] (35%)	1/8 (13%)	3/9 (33%)	32/228 (11%)
Lifestyle factors as most important contributor to obesity	n (%)	75 (27%)	1/19[†] (5%)	0/18[‡]	1/37[‡] (3%)	0/8	2/9 (22%)	72/228 (32%)
Socio-economic status z-score	Median (IQR) [min]	-0.1 (-1.2 - +0.5) [-4.8]	0.0 (-1.0 - +0.5) [-2.6]	-0.3 (-1.2 - +0.3) [-1.8]	0.0 (-1.0 - +0.4) [-2.6]	-0.2 (-1.1 - +1.1) [-3.5]	-0.4 (-1.3 - +0.4) [-3.3]	-0.1 (-1.4 - +0.5)[-4.8]
Short stature	n (%)	11 (4%)	0/19	4/18[‡] (22%)	4/37 (11%)	0/8	0/9	7/228 (3%)
Tall stature	n (%)	60 (21%)	6/19 (32%)	1/18 (6%)	7/37 (19%)	0/8	0/8	53/228 (22%)

ID, intellectual disability; VUS, variant of unknown significance; CNV, copy number variation; VUS, variants of uncertain significance; IQR, interquartile range; max, maximum; SD(S), standard deviation (score); BMI, body mass index; min, minimum.

[†]P<0.05 versus no definite singular underlying medical diagnosis group; [‡] P<0.01 versus no definite singular underlying medical diagnosis group.

Underlying medical causes of obesity

An underlying medical cause of obesity was identified in 54/282 (19.1%) patients in our cohort: 37 genetic obesity disorders, 9 medication-induced obesities, and 8 obesities due to cerebral injury (Table 1). None of the patients' obesity was explained by clinical hypothyroidism or Cushing's disease. In the remaining 228/282 (80.9%) patients no singular underlying medical cause of obesity could be identified. In 17 of these 228 patients a VUS/CNV was identified that possibly explains the obesity phenotype, but this still requires further research, such as functional studies, and therefore falls beyond the scope of this article.(24)

Genetic causes

Of the 37 patients with genetic obesity, 18 patients had a genetic obesity disorder with ID, and 19 without ID. Pathogenic variants in *MC4R* were the most commonly found genetic obesity disorder in our cohort and were found in 9/37 patients, corresponding to 3.2% of the total cohort of 282 patients. The second frequently identified genetic obesity disorders were biallelic *LEPR* pathogenic variants (6/37), followed by *GNAS* pathogenic variants leading to pseudohypoparathyroidism type 1a (5/37). The specific genetic aberrations are presented in Table 2. The clinical phenotypes of all patients with genetic obesity are described in Tables 3 and 4. Although most patients with a genetic obesity disorder had a combination of clinical features typical of their genetic obesity disorder, most patients did not have the *complete* clinical phenotype as mentioned in the ES guideline (Tables (Tables 3 and Table 4). Most notably, 6 out of 18 patients who were diagnosed with a genetic obesity disorder that is typically associated with ID did not have ID or developmental delay (Table 3).

Table 2. Overview of genetic alterations in patients diagnosed with a genetic obesity disorder

Pt	Gene/CNV	Reference transcript	Genetic alteration	Inheritance
Genetic obesity disorders without ID				
1	*MC4R*	NM_005912.2	Heterozygous c.105C>A p.(Tyr35*)	M
2	*MC4R*	NM_005912.2	Homozygous c.216C>A p.(Asn72Lys)	n.p.
3	*MC4R*	NM_005912.2	Heterozygous c.105C>A p.(Tyr35*)	M
4	*MC4R*	NM_005912.2	Compound heterozygous c.446_450del p.(Phe149Tyrfs*9), c.644T>G p.(Met215Arg)	P and M both heterozygous
5	*MC4R*	NM_005912.2	Homozygous c.779C>A p.(Pro260Gln)	P and M both heterozygous
6	*MC4R*	NM_005912.2	Heterozygous c.913C>T p.(Arg305Trp)	*de novo*
7	*MC4R*	NM_005912.2	Heterozygous c.380C>T p.(Ser127Leu)	P
8	*MC4R*	NM_005912	Heterozygous c.750_751del p.(Ile251Trpfs*34)	n.p.
9	*MC4R*	NM_006147.2	Homozygous c.785del p.(Phe262Serfs*4)	n.p.
10	*LEPR*	NM_001003679.3	Compound heterozygous c.2168c>T p.(Ser723Phe), c.1985T>C p.(Leu662Ser)	P and M both heterozygous

11	*LEPR*	NM_001003679.3	Compound heterozygous c.2051A>C p.(His684Pro), c.2627C>A p.(Pro876Gln)	P and M both heterozygous
12	*LEPR*	NM_002303.5	Compound heterozygous c.1753-1dup p.?, c.2168C>T p.(Ser723Phe)	P and M both heterozygous
13	*LEPR*	NM_002303.5	Homozygous c.1604-8A>G p.? intronic pathogenic variant affecting splicing	P and M both heterozygous
14	*LEPR*	NM_002303.5	Homozygous c.3414dup p.(Ala1139Cysfs*16)	P and M both heterozygous
15	*LEPR*	NM_002303.5	Compound heterozygous c.1835G>A p.(Arg612His), c.2051A>C p.(His684Pro)	P and M both heterozygous
16	*PCSK1*	NM_000439.4	Heterozygous c.541T>C p.(Tyr181His)[a]	M
17	*POMC*	NM_001035256.1	Heterozygous c.706C>G p.(Arg236Gly)[a]	n.p.
18	*SIM1*	n/a	6q16.3 deletion (chr6:100.879.864–102.471.598), disrupting *SIM1*	*de novo*
19	*STX16* (PHP 1b)	NM_003763.5	Heterozygous microdeletion c.331-?_585 + ? p.?	M
Genetic obesity disorders with ID				
1	*GNAS* (PHP1a)	NM_001077488	Heterozygous c.85C>T p.(Gln29*)	M
2	*GNAS* (PHP1a)	NM_000516.4	Heterozygous c.794G>A p.(Arg265His)	M
3	*GNAS* (PHP1a)	NM_018666.2	Heterozygous c.665T>C p.(Met222Thr)[b]	M and PM
4	*GNAS* (PHP1a)	NM_018666.2	Heterozygous c.665T>C p.(Met222Thr)[b]	M and PM
5	*GNAS* (PHP1a)	NM_018666.2	Heterozygous c.665T>C p.(Met222Thr)[b]	M and PM
6	16p11.2del	n/a	Distal 16p11.2 deletion (chr16:28,825,605–29,043,450, incl. *SH2B1*)	P and MP
7	16p11.2del	n/a	Distal 16p11.2 deletion (chr16:28,819,029–29,043,973,incl. *SH2B1*)	*de novo*
8	16p11.2del	n/a	Proximal 16p11.2 deletion (chr16:29,563,985–30,107,008, not incl. *SH2B1*)	*de novo*
9	mUPD14 (Temple syndrome)	n/a	Temple syndrome (caused by maternal uniparental disomy chromosome 14)	n/a
10	mUPD14 (Temple syndrome)	n/a	Temple syndrome (caused by maternal uniparental disomy chromosome 14)	n/a
11	*Epigenetic error chr14* (Temple syndrome)	n/a	Temple syndrome (caused by imprinting defect on chromosome 14)	n/a
12	*Epigenetic error chr14* (Temple syndrome)	n/a	Temple syndrome (caused by imprinting defect on chromosome 14)	n/a
13	*MKKS* (Bardet-Biedl syndrome)	NM_018848.3	Compound heterozygous c.110A>G p.(Tyr37Cys), c.950_960del p.(Gly317Aspfs*6)	P and M both heterozygous
14	*IFT74* (Bardet-Biedl syndrome)	NM_025103.3	Compound heterozygous c.371_372del p.(Gln124Argfs*9), c.16850-1G>T p.?	P and M both heterozygous
15	*MYT1L*	NM_015025.2	Heterozygous c.808del p.(Gln270Lysfs*11)	*de novo*
16	*POMC*	n/a	2p deletion (chr2:22.791.486–27.942.764), containing *POMC*)	*de novo*

17	*SPG11* (Spastic paraplegia 11)	NM_025137.3	Compound heterozygous c.4534dup p.(Asp1512Glyfs*7), c.5867-?_6477+?del p.? (deletion of exons 31–34	P and M both heterozygous
18	*VPS13B* (Cohen syndrome)	NM_017890.4	Compound heterozygous c.2911C>T p.(Arg971*), c.8697-2A>G p.?	P and M both heterozygous

CNV, copy number variation; SDS, standard deviation score; BMI, body mass index in kg/m2; ID, intellectual disability; M, mother; P, father; n.p., segregation analysis not performed; PHP 1b, pseudohypoparathyroidism type 1b; PHP 1a, pseudohypoparathyroidism type 1a; PM, father of mother; MP, mother of father; n/a, not applicable. [a]important genetic risk factor contributing to severe early-onset obesity; [b]siblings.

Table 3. Clinical characteristics of patients diagnosed with a genetic obesity disorder with ID

Gene/ CNV	***GNAS* (*PHP1a*)**	**16p11.2 deletion syndrome**	**Temple syndrome**	***MKKS* (Bardet-Biedl syndrome)**	***IFT74* (Bardet-Biedl syndrome)**	***MYT1L***	**2p-deletion syndrome**	***SPG11* (Spastic paraplegia 11)**	***VPS13B* (Cohen syndrome)**
Genetic cause*	Heterozygous disease-associated variant	16p11.2 deletion	Maternal uniparental disomy or imprinting defect of chromosome 14	Compound heterozygous disease-associated variants	Compound heterozygous disease-associated variants	Heterozygous disease-associated variant	2p-deletion syndrome, incl. *POMC*	Compound heterozygous disease-associated variants	Compound heterozygous disease-associated variants
Number of patients	5	3	4	1	1	1	1	1	1
Age at diagnosis in years, median (range)	11.6 (3.7–14.8)	6.6 (4.2–15.3)	9.8 (5.0–15.1)	1.7	11.2	3.3	12.8	14.0	4.4
Clinical features at initial visit									
Age in years, range	3.7–14.8	4.2–15.8	8.1–15.1	4.6	8.9	5.5	14.6	11.2	8.5
Height SDS, median (range)	-1.0 (-2.2 –- 0.5)	+0.9 (-2.4 – +1.5)	-1.0 (-2.1 – +1.1)	+0.7	+1.5	-0.6	-1.2	+1.4	-0.7
Δ Height SDS vs target height SDS, median (range)	-0.6 (-2.1 – +0.8)	+0.9 (-0.7 – +1.6)	-1.1 (-2.2 – +1.6)	+0.3	+0.9	0.0	0.0	+2.3	-0.7
BMI, median (max)	20.9 (27.1)	29.4 (30.1)	31.2 (33.4)	25.2	24.6	19.6	32.5	27.7	20.6
BMI SDS, median (max)	+1.8 (+3.6)	+2.8 (+5.3)	+3.3 (+3.5)	+5.5	+3.0	+2.5	+3.3	+3.4	+2.6
Early-onset <5 years	5/5	1/3	2/4	Yes	No	Yes	Yes	Yes	No
Hyperphagia	1/5	2/3	¾	No	No	Yes	Yes	Yes	No
ID	5/5	1/3	¼	Too young for formal testing; not suspected	No	Yes	Yes	Yes	Yes
History of abnormal neonatal feeding behavior	No	No	Hypotonia/feeding problems 4/4	Reduced satiety 1/1	Reduced satiety 1/1, resolved after infancy	No	No	No	Hypotonia/feeding problems 1/1

Clinical features characteristic of the genetic obesity disorder as mentioned in the Endocrine Society Guideline	Short stature in some but not all patients 1/5	Hyperphagia 2/3	*Genetic obesity syndrome not mentioned in guideline*	Developmental delay 1/1	Developmental delay 0/1	*Genetic obesity syndrome not mentioned in guideline*	*Genetic obesity syndrome not mentioned in guideline*	*Genetic obesity syndrome not mentioned in guideline*	*Genetic obesity syndrome not mentioned in guideline*
	Skeletal defects[b] 4/5	Disproportionate hyperinsulinemia 0/3		Dysmorphic extremities[c] 1/1	Dysmorphic extremities[c] 1/1				
	Impaired olfaction 0/5	Early speech and language delay 2/3 that often resolves 0/3		Retinal dystrophy or pigmentary retinopathy 1/1	Retinal dystrophy or pigmentary retinopathy 1/1				
				Hypogonadism n/a (due to young age)	Hypogonadism -				
	Hormone resistance (e.g., parathyroid hormone) 5/5	Behavioral problems including aggression 0/3		Renal abnormalities/impairment 1/1	Renal abnormalities/impairment 0/1				
Additional clinical features characteristic of the genetic obesity syndrome	Subcutaneous calcifications 1/5	N/A	Neonatal hypotonia 4/4	N/A	N/A	ID 1/1	Clinical features depending on size and location of deletion including hyperphagia (1/1). Generally no proopiomelanocortin	Progressive spastic paraplegia 1/1	Failure to thrive in childhood 1/1
						Autism 0/1		ID 1/1	Hypotonia 1/1
						Behavioral problems 0/1		Peripheral neuropathy 0/1	Microcephaly 1/1
									Visual impairment 1/1
			Neonatal feeding difficulties 4/4						Neutropenia 1/1

	Round facies 3/5		Short stature 2/4 Precocious puberty 4/4 Mild intellectual disability 2/4				deficiency (0/1). Additionally in our patient: ID, coarse facies with large front teeth		Prominent central incisors/ uplifted upper lip 1/1
Presence of genetic alteration in parents	All inherited from mother	1 inherited from father, 2 *de novo*	N/A	Both parents heterozygous	Both parents heterozygous	*De novo*	*De novo*	Both parents heterozygous	Both parents heterozygous
Presence of obesity in parents who carry the genetic alteration	Obesity *not* present	Obesity *not* present	N/A	Obesity *not* present (not associated with heterozygosity)	Obesity present in father (not associated with heterozygosity)	N/A	N/A	Obesity present in mother (not associated with heterozygosity)	Obesity present in father (not associated with heterozygosity)

CNV, copy number variation; SDS, standard deviation score; BMI, body mass index; ID, intellectual disablility; N/A, not applicable; PTH, parathyroid hormone; TSH, thyroid-stimulating hormone. *exact genetic alterations are listed in Table 2. [a]history of abnormal neonatal feeding behavior, i.e. reduced satiety and/or hypotonia/feeding problems; [b]skeletal defects, i.e. short metacarpalia dig IV and V (hands and/or feet); [c]dysmorphic extremities, e.g. syndactyly/brachydactyly/polydactyly, in our patients polydactyly.

Table 4. Clinical characteristics of patients diagnosed with a genetic obesity disorder without ID

Gene/CNV	***MC4R***		***LEPR***	***POMC***	**6q16.3 deletion**	***PCSK1***	***STX16* (PHP1b)**
Genetic cause*	Homoyzygous/compound heterozygous disease-associated variants	Heterozygous disease-associated variant	Homoyzygous/compound heterozygous disease-associated variants	Heterozygous disease-associated variant	6q16.3 deletion incl. part of *SIM1*	Heterozygous disease-associated variant	Heterozygous disease-associated variant
Number of patients	4	5	6	1	1	1	1

Age at diagnosis in years, median (range)	9.2 (1.6–15.4)	7.1 (2.2–15.3)	3.9 (0.7–14.8)	10.0	9.1	11.8	14.8
Clinical features at initial visit							
Age in years, range	6.5–15.4	2.5–15.3	0.7–17.7	10.0	9.1	12.2	17.2
Height SDS, median (range)	+0.8 (+0.7 –+2.2)	+2.1 (0.0 – +4.2)	+1.0 (-1.2 –+2.2)	-0.2	+3.0	-0.2	-0.1
Δ Height SDS vs target height SDS, median (range)	+1.4 (+0.7 –+3.2)	+0.7 (-0.1 – +4.1)	+1.2 (-1.3 –+1.5)	-0.5	+2.4	+1.0	-0.6
BMI, median (max)	34.0 (41.5)	27.9 (38.6)	35.3 (47.5)	28.2	36.8	32.9	31.4
BMI SDS, median (max)	+4.3 (+5.2)	+4.2 (+5.4)	+4.6 (+8.9)	+3.9	+4.4	+3.5	+2.9
Early-onset <5 years	3/4	5/5	6/6	Yes	Yes	Yes	Yes
Hyperphagia	3/4	5/5	5/6	No	Yes	No	Yes
ID	0/4	0/4	0/6	No	No	No	No
History of abnormal neonatal feeding behavior	Reduced satiety 3/4	No	Reduced satiety 4/6	No	No	No	Reduced satiety 1/1
Clinical features characteristic of the genetic obesity disorder as mentioned in the Endocrine	Hyperphagia 4/4	Hyperphagia 4/5	Extreme hyperphagia 5/6	*Genetic obesity syndrome not mentioned in guideline*	*Genetic obesity syndrome not mentioned in guideline*	*Genetic obesity syndrome not mentioned in guideline*	*Genetic obesity syndrome not mentioned in guideline*
	Accelerated linear growth 3/4	Accelerated linear growth 3/5	Frequent infections 0/6				
	Disproportionate hyperinsulinemia 4/4	Disproportionate hyperinsulinemia 1/5	Hypogonadotropic hypogonadism 3/4[c]				

Society Guideline	Low/normal blood pressure 1/4	Low/normal blood pressure 4/4[b]	Mild hypothyroidism 2/6				
Additional clinical features characteristic of the genetic obesity disorder	More severe phenotype than autosomal dominant	N/A	Growth hormone deficiency 1/6	Hyperphagia (less severe than autosomal recessive *PO MC* deficiency) 0/1	Characteristics depending on size of deletion: Intellectual disability 0/1	Hyperphagia (less severe than autosomal recessive *PCSK 1* deficiency) 0/1	PTH resistance 1/1;
							Occasionally partial TSH resistance 1/1
					Autism 0/1		Enhanced intrauterine growth 1/1
					Behavioral problems 0/1		Occasionally mild brachydactyly 1/1
							Round facies 1/1
Presence of genetic alteration in parents	2/4 both parents heterozygous 2/4 n.p.	3/5 inherited from parent, 1/5 *de novo*, 1/5 n.p.	All parents heterozygous	n.p.	*De novo*	Inherited from mother	Inherited from mother
Presence of obesity in parents who carry the genetic alteration	Obesity present in 1/4 heterozygous parents (known reduced penetrance)	Obesity present in 1 /3 heterozygous parents (known reduced penetrance)	Obesity present in 3/12 heterozygous parents (unclear association with heterozygosity)	N/A	N/A	Obesity present in heterozygous mother	Obesity *not* pres ent in heterozygous mother

PHP1b, pseudohypoparathyroidism type 1b; SDS, standard deviation score; BMI, body mass index; ID, intellectual disablility; BP, blood pressure; N/A, not applicable; PTH, parathyroid hormone; TSH, thyroid-stimulating hormone; n.p., not performed. *exact genetic alterations are listed in Table 2. [a]history of abnormal neonatal feeding behavior, i.e. reduced satiety and/or hypotonia/feeding problems; [b]In 1 patient, BP could not be measured due to unrest. [c]In 2 prepubertal patients not (yet) detectable.

In 3/37 cases, a heterozygous mutation/CNV was identified (in 2 patients in *POMC* and in 1 patient in *PCSK1*), which constitutes important genetic risk factors for early-onset obesity as demonstrated in association studies, in contrast to their autosomal recessive forms which cause a more severe clinical phenotype (S1 Appendix).(27, 39)

Cerebral injury as cause of obesity

We identified cerebral injury as the underlying medical cause of obesity in 8/282 (3%) patients. In five patients onset of rapid weight gain, objectified through analysis of their growth charts, coincided with intracranial surgery and/or radiotherapy (two craniopharyngiomas and three malignancies in the hypothalamic region). One patient had congenital anatomic midline defects in the hypothalamic region and clear hyperphagia and excessive weight gain from birth. In the remaining two patients onset of rapid weight gain occurred after meningitis or ischemic infarction, suggesting hypothalamic dysfunction.

Use of known weight-inducing medication as cause of obesity

In 9/282 patients (3%) medication-induced obesity was diagnosed through the combination of extensive evaluation of their growth charts and medication history and exclusion of endocrine, genetic, or cerebral causes of obesity. Of these nine patients, six were chronic users of inhalation corticosteroids (ICS). In 5/6 patients, periods of sudden weight gain, as seen on their growth charts, coincided with intermittent use of oral corticosteroids in the absence of other plausible causes of their sudden weight gain. In the remaining patient periods of intensification of chronic ICS use coincided with sudden weight gain according to the growth chart, without other plausible explanations for the sudden weight gain. In the other three patients the start and restart of antipsychotic drugs in one, and antiepileptic drugs in two patients, coincided with sudden weight gain.

<u>Comparison of phenotype in patients with genetic obesity disorders and patients without a singular underlying medical cause of obesity</u>

Patients with genetic obesity disorders more often had an extreme early-onset of obesity <5 years ($p = 0.04$) and hyperphagia ($p = 0.001$) when compared to patients without a singular underlying medical cause of obesity (Table 1, detailed p-values in S1 Table). Furthermore, the presence of obesity in parents ($p = 0.02$) and psychosocial problems (determined by the involvement of official authorities or DSM-V diagnosis; $p = = 0.001$) were less often present in the genetic obesity group. No significant differences were found with respect to BMI SDS, sex, socio-economic status z-score and family history of consanguinity or bariatric surgery (all $p>0.05$; detailed p-values in S1 Table). When zooming in on patients with genetic obesity with ID, they more often had short stature ($p = 0.005$), a history of neonatal feeding problems ($p = 0.003$), a dysmorphic appearance and/or congenital anomalies ($p<0.001$), and less severe obesity (lower BMI SDS; $p<0.001$) than patients without a singular underlying medical cause of obesity. Extreme early-onset obesity <5

years and hyperphagia were not present more often in the patients with genetic obesity disorders with ID (Table 1). With regard to height SDS, patients with genetic obesity without ID had a higher height SDS than patients without a singular underlying medical cause of obesity, although this difference was not statistically significant (p = 0.19). In contrast, patients with genetic obesity with ID had a significantly lower height SDS (p = 0.004).

Comparison of patients with cerebral or medication-induced obesities with other subgroups of patients

No assessed phenotype features were specifically present or absent in patients with cerebral or medication-induced obesities (Table 1). However, on a group level, these patients had lower height SDS than patients with genetic obesity disorders without ID or patients without underlying medical causes of the obesity.

Discussion

In this study, an extensive systematic diagnostic approach in a specialized obesity center established an underlying medical cause of obesity in 19% of pediatric patients. These included genetic obesity disorders (13%), medication-induced obesities (3%) and obesities due to cerebral injury (3%). To the best of our knowledge, this is the first study which reports the yield of a broad diagnostic workup in a tertiary pediatric obesity cohort, focusing not only on genetic obesity disorders but also on endocrine, medication-induced, and cerebral causes of obesity. Previously, Reinehr *et al*. assessed the prevalence of endocrine causes and of specific genetic causes, namely clinically identifiable syndromal causes and *MC4R* pathogenic variants in a subgroup of their cohort.(7) Their study, performed in 1405 children and adolescents visiting a specialized clinic for endocrinology and obesity, demonstrated an underlying disorder in 13 (1.7%) patients.

There are some explanations for our high diagnostic yield. First, our patients constitute a tertiary pediatric obesity population with severe obesity who were referred because of a suspicion of an underlying medical cause, or resistance to lifestyle interventions. Thus, we had a higher *a priori* probability of finding underlying medical causes than in an unselected pediatric obesity population. Nevertheless, we show that a broad systematic diagnostic workup is needed to identify these diverse underlying causes of obesity. Secondly, medication use and cerebral/hypothalamic injury were not mentioned in the evaluation of other cohorts, although they are part of the recommended diagnostic workup of the ES guideline for pediatric obesity.(13) Furthermore, the guideline mentions only antipsychotics as weight-inducing medication, but we also considered specific antipsychotic or anti-epileptic drugs and prolonged use of corticosteroids as potential cause of obesity in individual patients, but only in the presence of a temporal relationship with onset of obesity, objectified through comprehensive growth chart analysis, and in the absence of other underlying medical causes of obesity or other plausible explanations for the sudden weight

gain.(35-38) Comprehensive growth chart analysis was also supportive in the identification of patients with cerebral/hypothalamic injury as the cause of their obesity in our cohort. Thus, future guidelines might benefit from adding growth chart analysis as part of the diagnostic workup of pediatric obesity. Thirdly, intellectual disability was present in 24% of patients, which increased the *a priori* probability of genetic obesity disorders with ID. The last explanation for our high yield is the extensive genetic testing we performed. Pathogenic variants in *MC4R* were the most frequently identified genetic cause of obesity in our cohort (9/282 patients, 3.2%). This number is comparable to previous findings in another Dutch tertiary pediatric cohort (2.1%) and 1.6–2.6% in other non-consanguineous pediatric cohorts screening for genetic obesity.(40-42) However, in many studies, only *MC4R* mutations or a small number of obesity-associated genes are tested.(7, 27, 40–43) In our cohort, 13 genetic obesity disorders other than *MC4R* were present. Thus, this study shows that extensive genotyping can highly augment the diagnostic yield when performed in similar pediatric obesity cohorts. The extent to which heterozygous mutations/CNV in *PCSK1* and *POMC* are involved in monogenic obesity remains a point of discussion. Association studies clearly demonstrate that these rare variants contribute to a highly increased risk for obesity.(27, 39) Moreover, identifying these patients is of clinical importance for patient-tailored treatment as clinical trials with MC4R-agonist setmelanotide will be conducted, as it is hypothesized that these patients will have reduced MC4R functioning.(44)

We did not identify patients with an endocrine disorder as the cause of obesity. None of the patients were diagnosed with Cushing's syndrome. Pediatric Cushing's syndrome is extremely rare, and patients are often referred due to impaired growth velocity and abnormal laboratory results.(45, 13) Therefore, in contrast to adults, these patients are not primarily referred to obesity clinics. Retrospective analysis of ICD-10 codes for Cushing's syndrome in the central hospital registries at both participating centers during the entire study period (2015–2018) showed four diagnoses of pediatric Cushing's syndrome in these years; none of these four patients developed severe obesity. Importantly, PWS, the most common genetic obesity disorder with ID, was not identified in our cohort. This can be explained by the fact that in Dutch pediatric practice, PWS is often diagnosed during the neonatal period due to the typical hypotonia and feeding problems and after diagnosis, clinical care is transferred to specialized PWS expertise centers.

The second aim of our study was to present the phenotype of patients with underlying medical causes and investigate whether they can be distinguished from patients without underlying medical causes. We therefore performed the comprehensive diagnostic workup in all patients. In daily clinical practice with lower *a priori* probability of underlying medical causes, it is complex to determine for whom these diagnostics should be performed. According to literature, one of the most important features to help distinguish these

patients is their stature. Reinehr *et al*. reported that short stature had a high sensitivity for underlying causes of obesity in their cohort.(7) In our study, patients with genetic obesity disorders associated with ID, and patients with cerebral and medication-induced obesities in our cohort indeed had lower height SDS than expected based on the fact that obesity is associated with taller stature.(46) However, most of these patients did not fulfill the definition for short stature.(19) Unsurprisingly, cardinal features of genetic obesity disorders, namely early onset of obesity (<5 years) and hyperphagia, were more often present in patients with genetic obesity, but only when ID was not present. On the other hand, patients with genetic obesity disorders with ID more often had a history of neonatal feeding problems and congenital anomalies or dysmorphic features. Thus, presence of these features should lead to consideration to perform additional diagnostics. Contrary to expectations BMI SDS was not significantly higher in patients with genetic obesity compared to patients without underlying medical causes. A possible explanation is that severity of obesity increases the probability of being referred to a pediatric obesity center regardless of whether genetic obesity is diagnosed. Important factors that were more frequently present in the patients without underlying medical causes were psychosocial problems (DSM-5 diagnosis or involvement of authorities such as child protective services). These psychosocial problems might contribute to developing a higher BMI SDS.(47) On group level, we did not find evidence for significant differences in socio-economic status scores between patients with genetic obesity and patients without underlying medical causes, but individual differences in socio-economic factors and obesogenic environments might also play a role. Interestingly, parents of children with a genetic obesity disorder more often had no obesity than parents of children without an underlying cause. This sounds counterintuitive for hereditary obesity disorders, but can be explained by the fact that most of the genetic aberrations in our cohort had occurred *de novo* or had an autosomal recessive inheritance pattern. Thus, negative family history of obesity could therefore suggest a genetic obesity disorder. In conclusion, we show that several phenotypic features differed significantly between patients with and without underlying medical causes of obesity, but no feature was specific. Thus, a broad diagnostic workup is warranted in patients with a high suspicion of an underlying medical cause of obesity, e.g., in cases with early-onset obesity, hyperphagia, relatively low height SDS (especially in the presence of ID) and presence of sudden weight changes objectified through comprehensive growth chart analysis.

Treatment of multifactorial disorders such as obesity is complex. In our approach, all patients received a multidisciplinary treatment advice tailored to their personal needs, including personalized dietary and physical activity advice (Fig 2). Furthermore, a monitoring and follow-up plan was developed for every patient. Local health care providers, including child health clinic physicians, general practitioners, general pediatricians, and psychologists, were contacted for local implementation of the care plan. In cases with severe hyperphagia, parental support by an educational therapist was offered to cope with

the child's behavior. Rehabilitation physicians were consulted when obesity interfered with performance of daily activities such as walking.(10)

Establishing a main underlying cause of obesity can improve personalized treatment.(34) In all our 54 patients with an underlying medical cause, counseling about the diagnosis was given. This included advice pertaining to bariatric surgery, which has unclear long-term success rates for patients with underlying medical causes.(43, 48) Patients with genetic obesity were counseled by a clinical geneticist regarding inheritance, associated medical problems and reproductive decisions. Hormonal supplementation was started in case of hormonal deficiencies associated with specific genetic obesity disorders (such as growth hormone treatment in cases with leptin receptor deficiency).(49) In cases of syndromic obesity, the patients were evaluated for associated organ abnormalities or referred for disease-specific surveillance.(13, 25–32) In patients with cerebral/hypothalamic injury as cause of obesity and hyperphagia, dexamphetamine treatment was considered.(50) In patients with medication-induced obesity, evaluation of necessity and alternatives for the weight-inducing medication took place in collaboration with the prescribing physician. Follow-up studies are necessary to evaluate the different individual responses to these treatment options. Interesting novel developments are clinical trials with MC4R-agonists in patients with leptin-melanocortin pathway deficiencies, e.g. *POMC* and *LEPR* deficiency, and glucagon-like peptide 1 (GLP-1) agonists for adolescents with obesity.(44, 51) These GLP-1 agonists might also be a future treatment option for patients with genetic obesity disorders, as they have been shown to be equally as effective in adults with heterozygous *MC4R* mutations compared to adults without.(52) Recently, it was suggested that a subgroup of patients with severe early-onset obesity might have relative leptin deficiency and therefore might benefit from recombinant leptin administration.(53) However, the (long-term) effects of these new potential treatment options remain to be investigated.

Strengths and limitations

A major strength of our study is the use of a systematic diagnostic strategy in all patients investigating all medical causes of obesity mentioned in the current international guideline.(13) Moreover, we performed genetic diagnostics in all patients, and further genetic tests when clinically indicated. Furthermore, our relatively high diagnostic yield enabled us to describe the clinical phenotypes of a large number (n = 54) of patients with underlying causes of obesity from a relatively small patient cohort of 282 patients. When performing research in a diagnostic setting, one faces logistical limitations. During our study, three different versions of the diagnostic obesity-associated gene panel test were successively available for clinical use in The Netherlands. Importantly, in all used gene panels at least the most important and well-known obesity-associated genes were tested, including among others *LEP*, *LEPR*, *MC4R*, *POMC*, *PCSK1*, *ALMS1*, *GNAS*, *SH2B1*, and *SIM1*.

A strength of our diagnostic setting is that we followed the current ACMG guidelines for variant calling, leading to stringent selection of only pathogenic and likely pathogenic variants for which evidence from validated functional studies and from control populations has already been incorporated.(24) Children and adolescents with a high suspicion of a genetic cause with negative genetic testing results should be viewed as 'unsolved cases', for which current genetic tests are not yet able to pinpoint a diagnosis. As the field of obesity genetics is progressing rapidly, very recently discovered obesity genes were not present in the used diagnostic gene panels.(54) Incorporating these obesity genes might have resulted in an even higher diagnostic yield. Moreover, newer techniques such as whole-genome sequencing will become more easily accessible and affordable in clinical practice and will likely lead to more genetic obesity diagnoses.

We understand that our comprehensive approach is not feasible in every clinical setting, but our data suggest that it has added value for selected patient groups. Prospective studies looking at predictors for underlying medical causes of obesity are necessary but are difficult to establish because of the rarity of these disorders and overlap with common obesity. International collaboration in large multicenter studies using a similar standardized comprehensive approach are required.

Conclusion

In conclusion, we show that a large variety of underlying medical obesity diagnoses can be established in pediatric patients with obesity in tertiary care setting when using a comprehensive diagnostic workup. Investigating endocrine, genetic, cerebral and medication-induced causes of obesity is needed for these patients to facilitate disease-specific and patient-tailored treatment. Further studies on predictors of underlying medical causes of obesity are needed to improve identification of these patients.

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SUPPLEMENTARY APPENDIX

1. Protocol Obesity Center CGG
2. Obesity gene panel sequencing details
3. Supplementary table
   S1 Table. P-value table for differences in clinical features between the genetic obesity disorders group and the lifestyle obesity group
4. Supplementary appendix references

1. Protocol Obesity Center CGG pediatric division

Background

Obesity Center CGG (Dutch: '*Centrum Gezond Gewicht*'; English: 'Centre for Healthy Weight') is a Dutch multidisciplinary national referral center for diagnostics and personalized treatment for patients with obesity. Since 2015 children and adolescents visiting the outpatient pediatric CGG clinics of the university medical center Erasmus MC-Sophia Children's Hospital have been included. From 2016 on two collaborating general hospitals (Maasstad Ziekenhuis and Franciscus Gasthuis) have also included patients. In the current study, patients from the general hospital Franciscus Gasthuis were not included in our data analysis, as they did not undergo the complete standardized diagnostic procedure. According to Dutch law, written informed consent was obtained from parents and children >12 years; for children below age 12 years oral assent was obtained. This also included separate consent forms for genetic testing.

Overview of the pathway of the pediatric division of obesity center CGG

1. Review of historical/referral data
2. Intake by pediatric endocrinologist
3. Anthropometric measurements and vital signs
4. Questionnaires
5. Physiotherapist consultation (only for patients at the academic center Erasmus MC-Sophia Children's Hospital)
6. Nutritional assessment (only for patients at the academic center Erasmus MC-Sophia Children's Hospital)
7. Biochemical and hormonal evaluation
8. Genetic testing
9. Development and implementation of the care plan
10. Evaluation of the care plan (follow-up after 1 year)

1. <u>Review of historical/referral data</u>

Based on information provided in the referral letter, the patient is referred to the outpatient clinic of the academic center Erasmus MC-Sophia Children's Hospital (referral indications:

suspicion of an underlying cause of obesity including genetic causes of obesity, complex medical history and obesity) or general hospitals Maasstad Ziekenhuis/Franciscus Gasthuis (referral indications: diagnostic evaluation of possible underlying causes as well as comorbidities of obesity, personalized therapeutic advice for non-genetic or non-cerebral causes of obesity, or participation in a combined lifestyle program).(1) When a patient referred to a general hospital required specific academic expertise, the protocol is completed at the academic center.

2. Intake by pediatric endocrinologist

All patients are seen by a pediatric endocrinologist or a pediatrician supervised by a pediatric endocrinologist. Extensive phenotyping is performed to identify underlying endocrine, genetic, cerebral, and medication-induced main causes of obesity. A complete medical history is taken according to the Dutch pediatric guideline for evaluation of children and adolescents with obesity, which includes evaluation of neonatal feeding behavior, current and past weight-inducing medication use, motor and intellectual development, dysmorphic features or congenital anomalies.(2) This intake visit is not only focused on possible underlying causes of obesity, but also evaluates general health and well-being, lifestyle factors influencing obesity, possible comorbidities, psychosocial circumstances, and other potential barriers for successful treatment.

3. Anthropometric measurements and vital signs

Physical examination is performed according to the Dutch guidelines on pediatric obesity.(2) A wall-mounted stadiometer is used to measure height in 0·1 cm increments. When a child is under the age of two years, recumbent length is measured using an infantometer. Sitting height is the vertical distance between the sitting surface and the top of the head. It is measured in 0·1 cm increments, using the wall-mounted stadiometer and the sitting surface. Weight is measured using a calibrated scale while the children are lightly clothed and standing without shoes. Body mass index (BMI) is calculated as weight/height in meters squared (kg/m^2). Parental height and weight are also measured when parents are present during the visit at the outpatient clinic; if not present, estimated height and weight of the parents are recorded. Waist circumference in centimeters (0·1 cm increments) is measured between the superior anterior iliac crest and below the lowest rib after normal expiration, with patients standing and unclothed. Occiptofrontal circumference (head circumference; HC) is measured where the largest measurement can be obtained using a flexible tape measure. HC is measured in centimeters (0·1 cm increments). For all measurements, age and sex-specific standard deviation scores (SDS) were calculated using the latest Dutch national growth study as external standard.(3)

Blood pressure is measured on the bared right arm with a digital sphygmomanometer while the patient is seated. Both feet are flat on the floor and the patient is asked not to move or

talk during the measurements. Blood pressure is measured twice, the mean is recorded in the patient file. If blood pressure is elevated (>140 mmHg systolic or >90 mmHg diastolic), measurements are repeated twice with short intervals in between. Age, height, and sex-specific standard deviation scores (SDS) are calculated based on the reference values of the American Academy of Pediatrics.(4) Palpated radial pulse is taken while the patient is seated, registering the number of beats in 30 seconds or digitally assessed by the sphygmomanometer.

All measurements are conducted by outpatient clinic assistants who were specially trained.

4. Questionnaires

Patients and/or their parents are asked to fill out the following Dutch questionnaires before or after the visit to the outpatient clinic focusing on physical exercise and fitness, eating behavior, sleep behavior, stress and quality of life:

- Dutch General Obesity Questionnaire(2)
- Dutch Exercise Behavior Questionnaire, in Dutch: '*Basis Vragenlijst Bewegen'*, BVB (5)
- Dutch Eating Behavior Questionnaire, DEBQ (6)
- Sleep Disturbance Scale for Children, SDSC (7)
- Perceived Stress Questionnaire, PSQ (8)
- Pediatric Quality of Life Inventory (PedsQL) 4.0 (9)

Data collected through the questionnaires are discussed in the multidisciplinary consultation (see under '9. Development and implementation of the care plan').

5. Physiotherapist consultation (only for patients at the academic center Erasmus MC-Sophia Children's Hospital)

In children and adolescents visiting the outpatient clinic of the academic center Erasmus MC-Sophia Children's Hospital either the Bruce protocol or the 6-minute walking test (6MWT) is performed under supervision of a pediatric physiotherapist.
The Bruce protocol is a standardized treadmill test with an increasing treadmill speed and incline.(10) Heart rate and perceived exhaustion are monitored. The test is stopped when the child is exhausted; the maximal endurance time (in minutes, one decimal) serves as criterion of exercise capacity. For children who are not able to perform the Bruce protocol, for example due to intellectual disability, the 6MWT is performed. This test measures how far the patient can walk on a flat track in the exercise room when walking as fast as possible for six minutes. The results of both tests are compared to the norms that have been developed for healthy children.(11-13) Findings are discussed in the multidisciplinary consultation (see below).

6. Nutritional assessment (only for patients at the academic center Erasmus MC-Sophia Children's Hospital)

The following nutritional assessment is performed for all children and adolescents visiting the outpatient clinic of the academic center Erasmus MC-Sophia Children's Hospital under supervision of a pediatric dietitian.

- Dietetics: patients or their parents are asked to complete a food diary, recording all foods and drinks consumed over 2 consecutive days. An estimation of the total daily calorie intake is made, as well as an assessment of eating patterns, portion sizes, dietary behavior, and micronutrient intake.
- Resting energy expenditure is measured by indirect calorimetry (Quark RMR, COSMED).
- Body composition (fat mass and fat-free mass) is measured by air displacement plethysmography (BOD POD, COSMED) and/or dual energy x-ray absorptiometry (DEXA).

Findings are discussed in the multidisciplinary consultation (see below).

7. Biochemical and hormonal evaluation

Peripheral blood for biochemical and hormonal evaluation is obtained following overnight fasting. Next, a standard oral glucose tolerance test (OGTT) of 1·75 g of glucose per kg body weight (maximum 75 g glucose in 200 ml water) is performed between 8am and 10am. Plasma glucose and insulin are measured at t=0 and at t=2 hours; insulin at t=2 hours is only measured for patients at the academic hospital. The homeostatic model assessment of insulin resistance (HOMA-IR) value is calculated, using a cut-off for insulin resistance of >3·16.(14) Additionally, at t=0 hemoglobin A1c (HbA1c), total cholesterol, low density lipoprotein (LDL) cholesterol, high density lipoprotein (HDL) cholesterol, triglycerides, alanine transaminase (ALAT), aspartate transaminase (ASAT), Gamma-Glutamyl Transferase (GGT), thyroid hormones (FT4, TSH), cortisol, leptin, insulin-like growth factor 1 (IGF-1), testosterone, anti-Müllerian Hormone (AMH), sex hormone-binding globulin (SHBG), androstenedione, dehydroepiandrosterone sulfate (DHEAS) and 25-hydroxyvitamin D are measured according to local lab standards. All blood analyses are performed at the local medical laboratories of participating hospitals, all of which are ISO 15189 accredited.

8. Genetic testing

The following genetic tests are included in the extensive diagnostic workup:

- Next-generation sequencing analysis of obesity associated gene panel
- SNP-microarray analysis

On clinical suspicion, specific additional diagnostic tests (e.g., Prader-Willi syndrome diagnostics, maternal uniparental disomy (UPD) 14 test, trio whole exome sequencing) are performed.

Further details on the genetic tests can be found in the supplemental paragraph 2 'Obesity gene panel sequencing details'.

9. Development and implementation of the care plan

At the academic center Erasmus MC-Sophia Children's Hospital, all relevant findings of the diagnostic workup are discussed in a multidisciplinary consultation featuring a pediatric dietitian, a pediatric physiotherapist, pedagogue and pediatric endocrinologist. In this multidisciplinary meeting, the patient-tailored care plan is developed. The care plan includes dietary and physical activity advice, medical treatment (e.g. regarding comorbidities) or referral to combined lifestyle intervention, parent support center, psychologist or psychiatrist. Subsequently, patients are invited to the outpatient clinic to discuss the findings and the care plan. Afterwards, the care plan is communicated to the patient's referrer, who is responsible for implementing the tailored treatment advices locally.

10. Evaluation of the care plan (follow-up after 1 year)

The follow-up visit takes place after at least 1 year and includes evaluation of the patient-tailored care plan during the past year, followed by the same questionnaires, anthropometric measurements, and biochemical and hormonal evaluations (excl. OGTT) as during the intake visit. The results of genetic testing are discussed at the follow-up visit, or earlier when a relevant genetic alteration is found that requires counseling by a clinical geneticist.

2. Obesity gene panel sequencing details

Obesity gene panel testing is offered to all children who are included in this study. Because of logistic reasons, there were three different tests available in The Netherlands in the time span of this study. The details of the three obesity gene panels are listed below. The identified variants were compared with in-house and public databases, including www.mc4r.org.uk, to exclude common neutral variants. All variants were analyzed using mutation interpretation software to investigate their (possible) clinical relevance . Variants were classified according to the guideline of The American College of Medical Genetics and Genomics (ACMG).(15) If possible, a variant of uncertain significance (VUS) or an unknown copy number variation (CNV) was further investigated by family segregation analysis to clarify the pathogenicity. GRCh37/hg19 was used as reference genome.

Obesity Gene panel UMC Utrecht (Department of Genetics, UMC Utrecht, The Netherlands, ISO15189 accredited). December 2014 – November 2016

Gene	OMIM-entry	Inheritance	Name of associated syndrome or further details about the disease association
ALMS1	606844	Autosomal recessive	Alstrom syndrome
ARL6	608845	Autosomal recessive	Bardet-Biedl syndrome
BBS1, *BBS2,* *BBS4,* *BBS5,* *BBS7,* *BBS9,* *BBS10,* *BBS12*	209901 606151 600374 603650 607590 607968 610148 610683	Autosomal recessive	Bardet-Biedl syndrome
BDNF	113505	Autosomal dominant	Obesity associated gene
CCDC28B	610162	Autosomal recessive	Bardet-Biedl syndrome
CEP290	610142	Autosomal recessive	Bardet-Biedl syndrome, Joubert syndrome, Meckel syndrome
CRHR2	602034	-	Corticotropin-releasing hormone receptor
FLOT1	606998		Link to cholesterol uptake
G6PC	613742	Autosomal recessive	Glycogen storage disease 1a, von Gierke disease
GNAS	139320	Autosomal dominant	Albright hereditary osteodystrophy
IRS1	147545	Autosomal dominant	Comorbidity gene: insulin receptor
IRS2	600797	Autosomal dominant	Comorbidity gene: insulin receptor
IRS4	300904		Comorbidity gene: insulin receptor
KIDINS220	615759	Autosomal dominant	SINO syndrome (spastic paraplegia, intellectual disability, nystagmus, obesity)
LEP	164160	Autosomal recessive	Leptin deficiency
LEPR	601007	Severe: autosomal recessive	Leptin receptor deficiency
LZTFL1	606568	Autosomal recessive	Bardet-Biedl syndrome, Joubert syndrome, Meckel syndrome
MAGEL2	605283	Autosomal dominant	Schaaf-Yang syndrome
MC3R	155540	Autosomal dominant	Obesity associated gene
MC4R	155541	Severe: autosomal recessive Moderate: autosomal dominant	Melanocortin 4 receptor deficiency
MCHR1	601751	-	Obesity associated gene
MKKS	604896	Autosomal recessive	Bardet-Biedl syndrome, McKusick-Kaufman syndrome
MKRN3	603856	Autosomal dominant	Precocious puberty, Prader-Willi region
MKS1	609883	Autosomal recessive	Bardet-Biedl syndrome, Joubert syndrome, Meckel syndrome
MRAP2	615410	Autosomal dominant	Obesity associated gene
NDN	602117	Isolated cases	Prader-Willi region
NTRK2	600456	Autosomal dominant	Obesity associated gene
PAX6	607108	Autosomal dominant	Aniridia and obesity
PCK1	614168	Autosomal recessive	Phosphoenolpyruvate carboxykinase deficiency, cytosolic
PCSK1	162150	Severe: autosomal recessive Moderate: autosomal dominant	Obesity with impaired prohormone processing

PHF6	300414	X-linked recessive	Borjeson-Forssman-Lehmann syndrome
POMC	176830	Severe: autosomal recessive Moderate: autosomal dominant	Obesity, adrenal insufficiency, and red hair due to POMC deficiency
PRKAR1A	188830	Autosomal dominant	Acrodysostosis 1, with or without hormone resistance Carney complex, type 1 Myxoma, intracardiac Pigmented nodular adrenocortical disease
PTEN	601728	Autosomal dominant	PTEN hamartoma tumor syndrome
5SIM1	603128	Autosomal dominant	Obesity associated gene
SNRPD2	601061	-	Obesity pathway gene
SNRPN	182279	Autosomal dominant	Prader-Willi region
SPG11	610844	Autosomal recessive	Spastic paraplegia 11
TBX3	601621	Autosomal dominant	Ulnar-mammary syndrome
THRB	190160	Autosomal dominant	Comorbidity gene: thyroid hormone receptor
TMEM67	609884	Autosomal recessive	COACH syndrome, Joubert syndrome Meckel syndrome, Nephronophtisis, modifier of Bardet Biedl syndrome
TRIM32	602290	Autosomal recessive	Bardet Biedl syndrome, Muscular dystrophy, limb girdle, autosomal recessive
TTC8	608132	Autosomal recessive	Bardet Biedl syndrome
TUB	601197	Autosomal recessive	Retinal dystrophy and obesity
WDPCP	613580	Autosomal recessive	Bardet Biedl syndrome

Next Generation Sequencing (NGS) was performed on a SOLiD 5500XL system (Life Technologies). Horizontal coverage of >99% was achieved. Because of low coverage in a part of the *POMC* gene, additional Sanger sequencing was performed for this gene to achieve >99% horizontal coverage. Further details are provided in Kleinendorst et al., 2018.(16)

Obesity Gene Panel VUmc (Department of Genetics, Amsterdam UMC, location VUmc, The Netherlands, ISO15189 accredited). November 2016 – March 2018

Exome sequencing test with a custom filter. Whole-exome capture was performed using SeqCap EZ MedExome (Roche NimbleGen). Sequencing was done on a HiSeq 2500 or Hiseq 4000 sequencer (Illumina) (paired-end 125 bp and 150 bp reads respectively).The analysis was restricted to variants in a predetermined virtual panel of 52 genes associated with obesity and comorbidities. These were the same 52 genes as in the Utrecht obesity gene panel. If the coverage of the *MC4R* gene was less than 30X, additional Sanger sequencing was performed.

Obesity Gene Panel AMC (Department of Genetics, Amsterdam UMC, location AMC, The Netherlands, ISO15189 accredited). March 2018 - present (inclusion for this study: August 2018)

Gene list: *ALMS1, BDNF, CPE, GNAS, LEP, LEPR, MAGEL2, MC3R, MC4R, NPY4R, PCSK1, PHF6, POMC, SH2B1, SIM1, and VPS13B.*

Targeted enrichment was performed with custom in solution captures (SeqCap EZ Choice, Nimblegen). Sequencing was done on a MiSeq sequencer (Illumina) (paired-end 150 bp reads). All genes had a coverage of >30X. The analysis included CNV detection based on the NGS data. Sequences on chromosome 16p11.2 were included on the capture to allow for detection of a 16p11.2 deletion.

3. Supplementary table

S1 Table. P-value table for differences in clinical features between the genetic obesity disorders group and the patients without a singular underlying medical diagnosis

			Genetic obesity disorders without ID n=19	Genetic obesity disorders with ID n=18	Total genetic obesity disorders group n=37	Total no definite singular underlying medical diagnosis group n=228	P-value genetic without ID vs no definite singular underlying medical diagnosis	P-value genetic with ID vs no definite singular underlying medical diagnosis	P-value total genetic vs no definite singular underlying medical diagnosis
Age at initial visit		Median (IQR) [max]	10.0 (2.9-14.6) [17.7]	11.2 (7.1-14.7) [16.3]	10.0 (6.0-14.6) [17.7]	10.7 (7.7-13.6) [18.0]	P=0.32 (3)	P=0.81 (3)	P=0.36 (3)
Female		n (%)	14/19 (74%)	12/18 (67%)	26/37 (70%)	129/228 (57%)	P=0.15 (1)	P=0.41 (1)	P=0.12 (1)
Early-onset <5 years		n (%)	18/19 (95%)	12/18 (67%)	30/37 (81%)	146/228 (64%)	**P=0.006 (1)**	P=0.82 (1)	**P=0.04 (1)**
Hyperphagia		n (%)	15/19 (79%)	9/18 (50%)	24/37 (65%)	84/228 (37%)	**P<0.001 (1)**	P=0.27 (1)	**P=0.001 (1)**
Height SDS		Mean (SD) [max]	+1.1 (1.4) [+4.2]	-0.4 (1.3) [+1.5]	+0.3 (1.5) [+4.2]	+0.6 (1.3) [+3.7]	P=0.19 (4)	**P=0.004 (4)**	P=0.36 (3)
Weight SDS		Mean (SD) [max]	+4.6 (1.5) [+7.0]	+2.3 (1.5) [+5.2]	+3.5 (1.9) [+7.0]	+3.8 (1.1) [+7.1]	**P=0.04 (4)**	**P<0.001 (4)**	P=0.29 (3)
BMI SDS		Median (IQR) [max]	+4.2 (+3.5 - +4.7) [+8.9]	+3.1 (+2.4 - +3.5) [+5.5]	+3.5 (+2.8 - +4.4) [+8.9]	+3.8 (+3.3 - +4.3) [+6.6]	P=0.09 (4)	**P<0.001 (4)**	P=0.52 (3)
Head circumference SDS		Mean (SD) [max]	+2.0 (1.2) [+3.9]	+0.9 (1.5) [+3.8]	+1.4 (1.5) [+3.9]	+1.4 (1.1) [+4.9]	P=0.09 (4)	P=0.20 (4)	P=1.00 (3)

History of neonatal feeding problems		n (%)	0/19	5/18 (28%)	5/37 (14%)	11/228 (5)	P=1.00 (2)	**P=0.003 (3)**	P=0.06 (2)
ID		n (%)	0/19	12/18 (67%)	12/37 (32%)	48/228 (21%)	**P=0.03 (2)**	**P<0.001 (2)**	P=0.13 (1)
Autism		n (%)	1/19 (5%)	2/18 (11%)	3/37 (8%)	32/228 (14%)	P=0.48 (2)	P=1.00 (2)	P=0.44 (2)
Parents with obesity		n (%)	10/19 (53%) of which 1 both	9/18 (50%)	19/37 (51%) of which 1 both	161/228 (70%) of which 66 both	P=0.10 (1)	P=0.07 (1)	**P=0.02 (1)**
Parents with history of bariatric surgery		n (%)	1/19 (5%) 1 M	1/18 (6%) 1 M	2/37 (5%)	30/228 (13%) of which 3 both	P=0.48 (2)	P=0.71 (2)	P=0.28 (2)
Consanguinity		n (%)	2/19 (11%)	0/18	2/37 (5%)	20/228 (9%)	P=0.68 (2)	P=0.38 (2)	P=0.75 (2)
Psychosocial problems		n (%)	3/19 (16%)	4/18 (22%)	7/37 (19%)	115/228 (50%)	**P=0.004 (1)**	**P=0.02 (1)**	**P=0.001 (1)**
Current/past use of weight-inducing medication		n (%)	5/19 (26%)	2/18 (11%)	7/37 (19%)	59/228 (26%)	P=1.00 (2)	P=0.26 (2)	P=0.36 (1)
Evidently dysmorphic appearance and/or congenital anomaly		n (%)	1/19 (5%)	12/18 (67%)	13/37 (35%)	32/228 (11%)	P=0.48 (2)	**P<0.001 (2)**	**P=0.002 (1)**
Lifestyle factors as most important contributor to obesity		n (%)	1/19 (5%)	0/18	1/37 (3%)	72/228 (32%)	**P=0.02 (1)**	**P=0.005 (1)**	**P<0.001 (1)**

Socio-economic status z-score		Median (IQR) [min]	0.0 (-1.0 - +0.5) [-2.6]	-0.3 (-1.2 - +0.3) [-1.8]	0.0 (-1.0 - +0.4) [-2.6]	-0.1 (-1.4 - +0.5) [-4.8]	P=0.76 (4)	P=0.95 (4)	P=0.59 (3)
Short stature		n (%)	0/19	4/18 (22%)	4/37 (11%)	7/228 (3%)	P=1.00 (2)	**P=0.005 (2)**	P=0.052 (2)
Tall stature		n (%)	6/19 (32%)	1/18 (6%)	7/37 (19%)	53/228 (22%)	P=0.41 (2)	P=0.13 (2)	P=0.56 (1)
	ID, intellectual disability; IQR, interquartile range; max, maximum; SDS, standard deviation score; M, mother. (1) Chi squared test; (2) Fisher's exact test; (3) Independent sample t-test (if necessary, after log transformation). (4) Mann-Whitney U test. Cells in bold indicate a statistically significant difference between the mentioned groups.								

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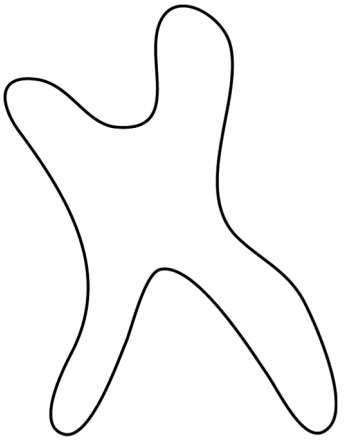
Chapter 5



Leptin receptor deficiency: a systematic literature review and prevalence estimation based on population genetics

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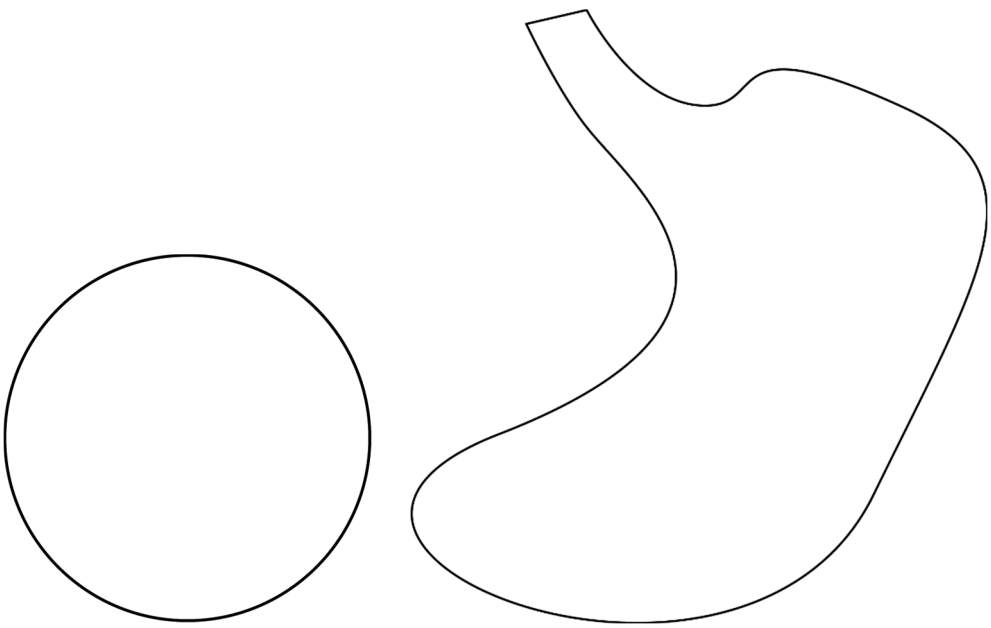

ABSTRACT

Objective Leptin receptor (LepR) deficiency is an autosomal-recessive endocrine disorder causing early-onset severe obesity, hyperphagia and pituitary hormone deficiencies. As effective pharmacological treatment has recently been developed, diagnosing LepR deficiency is urgent. However, recognition is challenging and prevalence is unknown. We aim to elucidate the clinical spectrum and to estimate the prevalence of LepR deficiency in Europe.

Design Comprehensive epidemiologic analysis and systematic literature review.

Methods We curated a list of *LEPR* variants described in patients and elaborately evaluated their phenotypes. Subsequently, we extracted allele frequencies from the Genome Aggregation Database (gnomAD), consisting of sequencing data of 77 165 European individuals. We then calculated the number of individuals with biallelic disease-causing *LEPR* variants. Results: Worldwide, 86 patients with LepR deficiency are published. We add two new patients, bringing the total of published patients to 88, of which 21 are European. All patients had early-onset obesity; 96% had hyperphagia; 34% had one or more pituitary hormone deficiencies. Our calculation results in 998 predicted patients in Europe, corresponding to a prevalence of 1.34 per 1 million people (95% CI: 0.95–1.72).

Conclusions This study shows that LepR deficiency is more prevalent in Europe (*n* = 998 predicted patients) than currently known (*n* = 21 patients), suggesting that LepR deficiency is underdiagnosed. An important cause for this could be lack of access to genetic testing. Another possible explanation is insufficient recognition, as only one-third of patients has pituitary hormone deficiencies. With novel highly effective treatment emerging, diagnosing LepR deficiency is more important than ever.

Introduction

Obesity is one of the most urgent health problems of modern times because of its epidemical prevalence, high disease burden, and high mortality.(1) In rare cases, obesity is caused by genetic disorders in the leptin-melanocortin pathway, the hypothalamic system controlling energy expenditure and food intake. The anorexic hormone leptin is mainly secreted by adipose tissue and reflects the body's energy reserves. Hypothalamic leptin signaling leads to activation of the melanocortin-4-receptor (MC4R), resulting in increased energy expenditure and satiety. When this signaling is disturbed, patients develop hyperphagia and early-onset obesity. A recent breakthrough for leptin-melanocortin pathway disorders is treatment with MC4R-agonist setmelanotide, which results in impressive weight loss.(2) One of the endocrine disorders that now can be treated is leptin receptor (LepR) deficiency, a rare autosomal recessive disorder caused by pathogenic variants in the leptin receptor gene (*LEPR*). Adequate functioning of the leptin receptor is essential for maintaining body weight. Moreover, adequate leptin signaling is necessary for onset of puberty, pubertal growth spurt, and production of thyroid-releasing hormone.(3, 4) Additionally, LepR-deficient rodents show decreased levels of pituitary growth hormone and stunted growth curves.(5)

When looking at the phenotype of LepR deficiency in humans, patients with LepR deficiency indeed can exhibit hypogonadotropic hypogonadism (HH), hypothyroidism, and/or growth hormone deficiency (GHD) in addition to extreme early-onset obesity and hyperphagia. It remains unclear why some patients only exhibit severe obesity, whereas others also have the associated pituitary hormonal disturbances. Residual receptor activity associated with specific *LEPR* mutations might partially explain this, but has not been investigated systematically.(4) Other features reported in patients with LepR deficiency are frequent infections and hyperinsulinemia, but to what extent they are part of the clinical spectrum of LepR deficiency is unknown.(3, 4) In some patients a lower CD4+ T-cell count and a compensatory higher B-cell count has been reported, which is in accordance with known effects of leptin on the immune system.(4) It is hypothesized that this may contribute to early childhood death due to infections.(4) Individuals affected by LepR deficiency have hyperinsulinemia to a degree consistent with the severity of their obesity, although it is suggested that these patients might be predisposed to develop insulin resistance and diabetes at an earlier age.(3, 4)

The phenotype variability makes identification of LepR deficiency challenging. Recognition might be further hampered due to lack of awareness of possible rare underlying causes in routine obesity care. In obesity cohort studies, LepR deficiency prevalence of 0–3% is found.(4, 6-8) Higher prevalence of up to 10% is reported in cohorts from consanguineous families.(9) However, it is important to realize that these estimations only reflect prevalence of LepR deficiency in selected patient groups. The traditional approach to prevalence

estimations of genetic diseases (counting the people diagnosed with the disease) greatly depends on local availability and application of genetic testing. Nowadays, genetic data from large population databases can be used to better estimate general prevalence of genetic disorders.

Aim of this study is to establish the prevalence of LepR deficiency in the general European population. To achieve this, we first performed a systematic literature review to identify all published cases and add unpublished cases from our obesity center. We use the *LEPR* variants from these cases to perform a prevalence estimation based on European allele frequencies. Our second aim is to gather clinical information from published LepR deficiency patients to describe the clinical spectrum.

Methods

Systematic literature search

A systematic literature search was performed in Embase, Medline (Ovid), Web of Science, Cochrane Library, and Google Scholar to identify all patients with LepR deficiency from its first report in 1998 up to May 2019. The complete search strategy is presented in the supplement (Supplementary file 1, see section on supplementary materialsgiven at the end of this article). In short, the strategy consisted of the themes '*LEPR*'/'LepR deficiency' or 'obesity genetic diagnostics'. We adopted a broad search strategy to not miss studies which sequenced *LEPR* as part of an obesity gene panel. Additionally, we searched for additional cases in ClinVar, the Human Gene Mutation Database, and the Decipher database.(10-12) Finally, we performed a non-systematic search in Researchgate (www.researchgate.net; accessed 24-05-2019; search queries 'LEPR', 'leptin receptor' and 'leptin receptor deficiency') to identify studies and conference abstracts that were not indexed in the mentioned databases.

Title and abstract of all identified studies were screened by two investigators (L K, O A); studies describing patients with LepR deficiency were included; duplicate studies were removed (Fig. 1). In case of disagreement over inclusion, a senior investigator (E v d A/M v H) served as adjudicator. Additionally, reference lists of included studies were screened for relevant articles. Follow-up studies on cases already described in literature were only used for phenotype assessment.

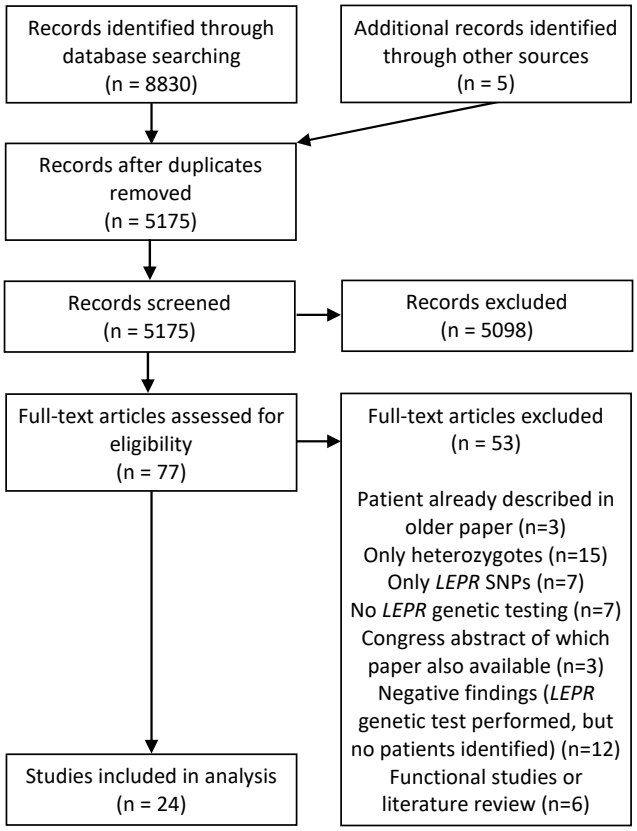

Figure 1. PRISMA flow diagram of systematic literature search
LEPR, leptin receptor gene, SNP, single nucleotide polymorphism.

Data extraction from included articles

An overview of genetic aberrations and phenotype features of patients with LepR deficiency (early-onset obesity, hyperphagia, signs of hypopituitarism and frequent infections) was made. When standard deviation scores (SDS) were not originally reported for anthropometric data, we calculated these using WHO growth charts as external standard.(13) In case insufficient clinical data were reported, we tried to contact corresponding authors to provide additional information.

Case presentation of Dutch patients with LepR deficiency

We present two novel cases of LepR deficiency identified in our obesity center. Written informed consent for publication of their clinical details was obtained from the patient and/or parents. For these two patients' anthropometric data, SDS are presented using Dutch growth charts as external standard.(14)

Selection of variants in LEPR

We extracted disease-associated *LEPR* variants from published cases identified through our systematic literature search and added in-house genetic data (Table 2). Additionally, we curated and added variants with a high likelihood of being pathogenic, that is, loss-of-function (LoF) variants that were proximal to the pathogenic variant p.S1090Wfs*6. This variant is the most distal pathogenic variant reported in a patient with LepR deficiency; hence, LoF variants located more proximally are very likely to cause LepR deficiency. For all selected *LEPR* variants, we extracted allele frequencies from the Genome Aggregation Database (gnomAD). The gnomAD database (https://gnomad.broadinstitute.org/; accessed 06-10-2019) is the largest freely accessible population-based database consisting of sequencing data from 77 165 Europeans. Individuals with known severe pediatric diseases and their first-degree relatives are removed from this database. We excluded variants that did not pass gnomAD's quality control. Because of their distinctive genetic background, Finnish individuals are often omitted from European population studies. However, by performing separate prevalence calculations for Finnish and Non-Finnish cases, we could aggregate the results and provide estimations for the whole European population. All selected *LEPR*variants were evaluated by a clinical laboratory geneticist according to the current international guideline for variant classification.(15) All variants are aligned to the canonical transcript NM_002303.5.

Prevalence calculation

We extracted European population size from the 2019 United Nations World Population Prospects report, which estimates a population size of 747.183 million Europeans, of which 5.532 Finnish Europeans(https://population.un.org/wpp/Download/Standard/Population/; accessed 28-09-2019). We estimated the number of individuals with biallelic (homozygous or compound heterozygous) pathogenic *LEPR* variants by calculating the probability of homozygosity or compound heterozygosity for each possible combination of our selected variants. We assumed that the population was in Hardy-Weinberg equilibrium and that random mating between individuals with and without obesity occurred. We did not correct for specific genetically isolated consanguineous populations in Europe. The CI of our prevalence estimation was calculated using derived variances.(16) We adapted the formulas to allow derivation based on the sum of independent random variables.

Results

Systematic literature search and overview of published cases

In total, 5175 records were screened (Fig. 1), of which 24 records presented unique patients with LepR deficiency and were eligible for inclusion (2, 4, 5, 7, 9, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35). From these 24 records, we identified *n* = 86 unique patients with LepR deficiency from 57 different families. We add two new unrelated cases with LepR deficiency (Box 1).

Box 1: Presentation of two new cases with LepR deficiency

The first patient is a 3-year-old boy, referred at age 13 months because of increased linear growth, obesity and hyperphagia. He was born at a gestational age of 36 + 6 weeks with normal birth weight (3840 g, +0.9 SDS). Parents did not report consanguinity, but their families lived in the same small Dutch municipality. There was no history of frequent infections. On presentation at age 13 months, height was 83.2 cm (+1.9 SDS), weight 17 kg (+4.9 SDS), and BMI 24.6 kg/m^2 (+4.4 SDS). Laboratory testing showed a central hypothyroidism. A growth hormone test was performed because of height deceleration, which confirmed GHD. Thyroid and growth hormone supplementation were started. Adrenal insufficiency was excluded by a high-dose ACTH test. MRI cerebrum revealed no anatomic abnormalities in the pituitary region. Obesity gene panel analysis (described in detail elsewhere (6)) revealed a homozygous variant of uncertain significance (VUS) in *LEPR*: c.3414dup p.(Ala1139Cysfs*16). This variant is located in the C terminal domain of the transcript. Since this is a frameshift near the end of the protein, replacing the last 27 amino acids with 15 alternative amino acids, the clinical relevance remains uncertain. However, the typical clinical phenotype (including hormonal disturbances) in the absence of other plausible explanations, makes this homozygous variant the most probable cause of the LepR deficiency phenotype.

The second patient is a 15-year-old girl referred to our obesity center at age 14 years for personalized treatment advice. She was born at a gestational age of 42 weeks with normal birth weight (3400 g, −0.1 SDS). At age 3.5 years, she was referred to a pediatric endocrinologist for evaluation of hyperphagia and obesity. There was no history of frequent infections. Height was 97 cm (−1.2 SDS), weight 23.1 kg (+3.0 SDS), BMI 24.6 kg/m^2 (+4.4 SDS). Laboratory testing showed no signs of hypopituitarism. During clinical follow-up, she had spontaneous start and progression of puberty and menarche at age 12.5 years. Whole-exome sequencing analysis revealed compound heterozygosity for two known pathogenic variants in the *LEPR* gene: c.1835G>A (p.Arg612His), c.2051A>C (p.His684Pro). Previously reported functional studies confirmed impaired functionality of the His684Pro variant, whereas the Arg612His variant has some residual function (4).

Including these two new cases, 88 patients have now been described worldwide (Table 1), harboring 45 distinct *LEPR* variants (Table 2). Twenty-one of these patients are from European ancestry. To gain more insight in the clinical spectrum of the disease, the phenotypes are summarized in Table 1 and presented on individual level in Supplementary Table 1 (which can be found at website of the European Journal of Endocrinology). Consanguinity was reported in 65/88 (74%) patients. Of the 84 patients in which sex was reported, 42 (50%) were female. Median age at description was 8.0 years (IQR: 3.0–15.2 years). Eighteen (22%) out of the 83 patients in which age was reported were adults, the

three oldest of which were 39, 41, and 55 years old. Median BMI was 39.6 kg/m^2 (IQR: 34.1–49.1 kg/m^2). Mean BMI SDS was +5.2 (SD 2.0) and was not significantly different between males and females (P = 0.39). Interestingly, three patients (Dehghani III:9 and III:10, Kakar VII:6) did not have obesity at presentation. A large inter-individual variation was seen with respect to height SDS (mean +0.3 SDS, s.d. 2.1; reported in 49/88 patients): 11/49 (22%) patients had a tall stature (height SDS >2), whereas 8/49 (16%) patients had a short stature (height SDS <−2). Early-onset obesity (<age 5 years) and hyperphagia were the most common phenotypic features (Table 1). In 21 cases, exact age of onset of obesity was reported; when aggregated, median age of onset was 0.3 years (IQR 0.2–0.4). Pituitary hormone disturbances were present in 24 patients (Table 1). In the majority of these patients (15/24, 63%), only one pituitary hormone disturbance was present. Three patients had both HH and GHD; one patient had HH and central hypothyroidism; one patient had GHD and central hypothyroidism. Three patients had HH, GHD as well as central hypothyroidism.

Known and likely pathogenic *LEPR* variants

Of the 45 distinct variants described in patients with LepR deficiency, only eight variants were present in the global gnomAD population, and seven were present in the European population of the gnomAD database (Table 2). Additionally, 20 LoF variants with a high likelihood of being pathogenic were identified in the European population of the gnomAD database (Supplementary Table 2). As expected, no (likely) pathogenic variants were present in a homozygous state in gnomAD.

Prevalence calculation

The calculated number of individuals with LepR deficiency (caused by biallelic disease-causing variants in the *LEPR* gene) in Europe is 998 patients (95% CI 708–1288). This would indicate that only 21/998 (2.1%) European cases with LepR deficiency are currently described in literature. The prevalence of LepR deficiency based on published European patients would be 0.03 per 1 million people. However, our calculated 'genetic prevalence' of LepR deficiency in Europe is 1.34 per 1 million people (95% CI 0.95–1.72 per 1 million people).

Table 1. Summarized overview of clinical characteristics of all 88 currently known patients with LepR deficiency

Features	*n* patients with available data (out of 88)	Interpretation
Early-onset obesity	87	Present in 87 (100%) patients: - 51 (59%) onset before age 2 years - 7 (8%) in (early) infancy - 5 (6%) onset between age 2–6 years - 1 (1%) onset before age 13–14 years - 23 (26%) not further specified
Hyperphagia	84	Present in 81 (96%) patients
Pituitary hormone disturbances	70	Present in 24 (34%) patients
Central hypothyroidism	64	Present in 8 (13%) patients
Growth hormone deficiency*	64	Present in 8 (13%) patients Additionally: - 3 (6%) IGF-1 values below reference range reported - 1 (2%) patients short stature reported
Hypogonadotropic hypogonadism	39	Present in 22 (56%) patients Additionally: - 1 (3%) inconclusive due to young age but low gonadotrophins reported
Hyperinsulinemia	61	Present in 24 (39%) patients Additionally: - 10 (16%) inconclusive because no reference range for insulin values was reported
Frequent infections	44	Present in 23 (52%) patients, of which 3 died due to infections in childhood Additionally: - 2 (5%) lowered CD4+ T cell count reported - 1 (2%) alterations in immune function reported

*Formal diagnosis of growth hormone deficiency by appropriate GH provocation tests. CD4, cluster of differentiation 4; IGF-1, insulin-like growth factor 1.

Table 2. Mutations in the *LEPR* gene described in patients with LepR deficiency

Reference	*n*	Nationality	Zygosity	Variant in coding DNA	Aberration on protein level (NM_002303.5)	Functional analysis	Allele frequency European non-Finnish population in gnomAD
(35)	1	N.R.	Hom	N.A.	p.M1?	N.R.	8.80E-06
(4)	3	Southern European	Hom	N.A.	p.W31*	N.A.	Not present
(26)	1	Turkish	Hom	c.461dupA	p.N154Kfs*3	*In silico*	Not present
(29)	9	Iranian	Hom	c.464T>G	p.Y155*	*In silico*	Not present
(22)	2	Sudanese	Hom	c.479delA	p.H160Lfs*10	*In silico*	Not present
(22)	1	Guinean	Hom	c.556delT	p.C186Afs*28	*In silico*	Not present
(17)	2	Egyptian		c.946C>A	p.P316T	*In silico*	1.76E-05
(18),(26)	2	Turkmen; Turkish	Hom	c.946C>A, c. 1938G>T (both hom)	p.P316T and p.W646C (both hom)	*In silico*	Not present
(4)	1	Turkish	Hom	c.1226C>A	p.A409E	*In vitro*	Not present

(24)	2	French	Comp het	c.1264T>C and c.2131dup	p.Y422H and p.T711Nfs*18	*In silico*	Not present
(32)	1	N.R.	Hom	c.1285+1G>A	p.? (splicing defect)	*In silico*	Not present
(31)	1	Turkish	Hom	c.1603+2T>C	p.? (splicing defect)	*In silico*	Not present
(21)	5	Pakistani	Hom	c.1603+5G>C	p.R468Sfs*33	*In silico*	Not present
(25)	1	Dutch	Hom	c.1604–8A>G	p.K536Sfs*34 and p.V535Dfs*3 (two transcripts)	*In silico*, Sanger, RNA analysis	8.92E-06
(24)	1	French (Reunion)	Comp het	c.1604–1G>A and del exon 6–8	p.? (splicing defect) and p.?	*In silico*	Not present
(23), (9)	2	Pakistani	Hom	c.1675G>A	p.W558*	*In silico*, Sanger	Not present
(25)	1	Dutch	Comp het	c.1753–1dupG and c.2168C>T	p.M585Dfs*2 and p.S723F	*In silico,*Sanger, RNA analysis	Not present
(24)	1	French	Hom	c.1810T>G	p.C604G	*In silico*	Not present
(9)	2	Pakistani	Hom	c.1810T>A	p.C604S	*In silico*	Not present
This publication	1	Dutch	Comp het	c.1835G>A and c.2051A>C	p.R612H and p.H684P	*In silico*	Not present
(4)	1	UK	Comp het	c.N.A. (1-bp deletion in codon 15) and c.1835G>A	p.F15Lfs*4 and p.R612H	*In vitro*(p.R612H)	Not present
(28)	1	Spanish	Hom	c.1835G>A	p.R612H	*In vitro*	4.88E-04
(35)	1	N.R.	Hom	c.1871dupA	p.N624Kfs*21	*In silico*	Not present
(26)	1	German	Comp het	c.1874G>A and c.2051A>C	p.W625* and p.H684P	*In silico*, *In vitro*(p.H684P)	Not present
(34)	3	Middle-eastern	Hom	c.1916C>T	p.P639L	*In silico*	Not present
(27)	1	Dutch	Comp het	c.1985T>C and c.2168C>T	p.L662S and p.S723F	*In silico*	Not present
(4)	1	Norwegian	Hom	N.A.	p.W664R	*In vitro*	5.31E-05
(4), (26)	2	UK; German	Hom	c.2051A>C	p.H684P	*In vitro*	3.87E-05
(7)	1	Dutch	Comp het	c.2051A>C and c.2627C>A	p.H684P and p.P876Q	*In silico*	Not present
(26)	1	German	Comp het	c.2227T>C and c.2598-3_2607delTAGAATGAAAAAG	p.S743P and p.Q865_K870	*In silico*	Not present
(24), (2)	2	Portuguese	Hom	c.2357T>C	p.L786P	*In silico*	8.82E-06

(23), (9)	4	Pakistani	Hom	c.2396-1G>T	p.? (splicing defect)	*In silico*	Not present
(24)	1	Turkish	Hom	c.2491G>A	p.H800_N831 del (splicing defect)	*In silico*	Not present
(5)	3	Algerian	Hom	c.2597+1G>A	p.? (splicing defect)	PCR and sequencing	Not present
(30)	1	Pakistani	Hom	c.2675C>G	p.P892R	*In silico*	Not present
(30)	4	Pakistani	Hom	c.3268_3269del	p.S1090Wfs*6	*In silico*	Not present
(33)	5	Indian	Hom	c.3268_3269dup	p.S1090Rfs*6	*In silico*	Not present
This publication	1	Dutch	Hom	c.3414dup	p.A1139Cfs*16	*In silico*	Not present
(19)	1	N.R.	Hom	deletion *DNAJC6* and parts of *LEPR*	p.?	PCR, MPLC	Not present
(9)	1	Pakistani	Hom	1.3 kb and 58.8 kb deletions	p.?	*In silico*	Not present
(26)	1	Turkish	Hom	deletion exon 4–20	p.?	N.A.	Not present
(4)	3	Bangladeshi	Hom	N.A. (4-bp deletion codon 22)	N.A.	*In silico*	Not present
(4)	2	Turkish	Hom	N.A. (11-bp deletion codon 70)	N.A.	*In silico*	Not present
(24)	5	French (Reunion)	Hom	deletion exon 6–8	p.?	*In silico*, PCR	Not present
(4)	1	Iranian	Hom	N.A. (66-bp deletion codon 514)	N.A.	*In silico*	Not present

bp, base pair; Comp het, compound heterozygous; del, deletion; gnomAD, Genome Aggregation database; Hom, homozygous; MPLC, multiplex polymerase chain reaction/liquid chromatography; n, number of patients; N.A., not applicable; N.R., not reported; PCR, polymerase chain reaction; UK, United Kingdom.

Discussion

Leptin receptor deficiency is a rare endocrine disease, but our population genetics-based analysis shows that it is much more prevalent in Europe than expected based on literature. Assuming that most patients with LepR deficiency have been published, as is demonstrated by the ongoing reports of new cases in the past years, this suggests underdiagnosis. This is especially problematic since diagnosing LepR deficiency now has therapeutic consequences: pharmacological treatment aimed at restoring the leptin-melanocortin pathway has recently shown impressive results in terms of weight loss, satiety, and improvement of metabolic parameters.(2)

Genetic testing for obesity disorders, including LepR deficiency, is recommended in patients with extreme early-onset (before age 5 years) and clinical features of a genetic obesity

disorder and/or a positive family history for extreme obesity.(36) However, a recent review from the United States reports that only 8% of patients in whom genetic testing would be indicated had undergone genetic testing.(37) An important reason for underdiagnosing might be limited access to genetic diagnostics. Although *LEPR* sequencing has become available in clinical practice in the last decade, it is not yet part of routine care in many countries. Indeed, all published European LepR deficiency cases are from high-income countries with well-established diagnostic genetic facilities. Another explanation why patients with LepR deficiency are not identified, is that the clinical phenotype is not sufficiently recognized. Our systematic literature search shows that the majority of patients do not have pituitary hormonal disturbances. It is hypothesized that there might be a genotype–phenotype correlation reflecting residual leptin receptor function in those cases, but the amount of patients is too small to draw conclusions.(3) Thus, LepR deficiency should be suspected in all cases of severe early-onset obesity and hyperphagia, even without signs of hypopituitarism, especially in the case of consanguinity. In the most common monogenic obesity disorder, *MC4R* deficiency, segregation studies have shown incomplete expressivity and penetrance for the obesity phenotype (38). However, this is not likely for LepR deficiency, as there are no individuals present with biallelic pathogenic *LEPR* variants in gnomAD nor in large control cohorts without obesity.(6, 39)

A more daunting possible cause of the discrepancy between amount of described patients versus predicted patients is mortality. Young age of known patients and absence of adult LepR deficiency patients in several large adult cohorts with early-onset obesity could suggest that these patients decease before they are identified.(6-8) This may occur due to the consequences of their severe obesity, but mortality in early childhood due to infections has also been reported.(4, 29) Long-term follow-up studies of the clinical course of LepR deficiency have however not yet been performed. These studies are also needed because in some cases, improvement of the endocrine phenotype after puberty has been reported, however, without a clear explanation. Le Beyec *et al*. reported resolving of central hypothyroidism from age 16 years onward and hypogonadism from age 19 years onward in a male patient.(20) Dehghani *et al*. reported that two affected males in a consanguineous family showed BMI normalization from puberty onset onward, in contrast to the affected females in this family who did not show improvement of BMI nor hypogonadotropic hypogonadism, suggesting a sex-specific effect might be present.(29) However, Nizard *et al*. reported resolving of hypogonadotropic hypogonadism in a female patient from age 18 years onward and occurrence of natural pregnancy 2 years after gastric bypass surgery, which challenges the assumption that hormonal disturbances only resolve in male patients.(40) However, the number of patients is too low to draw conclusions on this phenomenon.

Strengths and limitations

To the best of our knowledge, this is the first systematic literature overview of LepR deficiency cases. We identified 86 published cases, compared to the 57 cases in a previous, non-systematic overview from 2018.(3) A strength of this study is that we could add clinical information from 26/86 (30%) known LepR deficiency cases by contacting authors. Another strength is our stringent variant selection. There is always an insecurity regarding the pathogenicity of variants when functional tests have not been performed. This is even the case for variants identified in patients with clear LepR deficiency phenotypes, such as the male patient described earlier. In 2018, Ayers *et al*. presented a prevalence calculation for LepR deficiency in the United States.(41) However, they estimated prevalence using a far less stringent method by adding variants predicted to be pathogenic solely on the basis of *in silico* prediction tools. It is known that these tools are not specific, leading to high false-positive rates.(42) When we would use their method, this would lead to a prevalence estimation of 8953 patients (95% CI: 7880–10 027 patients). This would be a significant overestimation, whereas our calculation would rather yield an underestimation of actual number of patients. An important limitation of our study is that only 7/45 distinct pathogenic variants identified in patients with LepR deficiency were present in the European gnomAD population. Therefore, when sample size of sequencing data in population databases expands, prevalence calculations might yield a higher number of patients. Another limitation of our calculation is that first-degree relatives from patients with severe pediatric diseases, such as LepR deficiency, are removed from gnomAD, which could have led to a lower allele frequency of pathogenic *LEPR* variants. Moreover, we are aware that it is possible that some diagnosed patients have not been described in literature yet. This could lead to a higher prevalence calculation if these patients have novel *LEPR* variants. Thus, our current prevalence calculation should be seen as a minimum estimation.

Conclusion

LepR deficiency is an endocrine obesity disorder for which encouraging treatment options recently became available. Genetic testing in patients with early-onset obesity, hyperphagia, and/or LepR-associated hormone disturbances is therefore more important than ever. By using large population-based genetic data, we estimated the prevalence of this rare disease in Europe. Our data suggest that the majority of patients with LepR deficiency in Europe are currently not recognized. Improving awareness and availability of genetic testing for early-onset obesity is needed to help these patients gain access to newly developed effective treatment.

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SUPPLEMENTARY APPENDIX

Supplementary file 1. Search strategy for systematic literature search
Date of search: May 17th 2019

Embase – 2822 refs

(((('obesity'/exp OR 'body mass'/de OR 'body weight'/exp) AND ('leptin receptor'/de)) OR (((obes* OR BMI OR body-mass* OR weight* OR overweight*) AND (LEPR OR leptinreceptor* OR ((leptin* OR LEP) NEAR/3 (receptor*)))) OR (((obes* OR BMI OR body-mass* OR weight* OR overweight*) NEAR/5 (gene OR genes OR genom* OR mutat* OR genet* OR monogen* OR nonsyndrom*))) AND (exome* OR sequencing* OR delet* OR mutat* OR variant* OR splic*)):ab,ti) AND ('clinical study'/exp OR (clinical* OR case OR cases OR patient* OR cohort* OR male* OR female* OR man OR men OR woman OR women OR girl* OR boy* OR child*):ab,ti) NOT ((polymorph* OR SNP) NOT (mutation* OR exome* OR delet* OR splic*)) NOT ([animals]/lim NOT [humans]/lim) AND [english]/lim NOT ([Conference Abstract]/lim AND [1800-2016]/py)

Medline – 1990 refs

(((exp Overweight/ OR Body Mass Index/ OR Body Weight/) AND (Receptors, Leptin/)) OR (((obes* OR BMI OR body-mass* OR weight* OR overweight*) AND (LEPR OR leptinreceptor* OR ((leptin* OR LEP) ADJ3 (receptor*)))) OR (((obes* OR BMI OR body-mass* OR weight* OR overweight*) ADJ5 (gene OR genes OR genom* OR mutat* OR genet* OR monogen* OR nonsyndrom*))) AND (exome* OR sequencing* OR delet* OR mutat* OR variant* OR splic*)).ab,ti.) AND (exp Clinical Study/ OR (clinical* OR case OR cases OR patient* OR cohort* OR male* OR female* OR man OR men OR woman OR women OR girl* OR boy* OR child*).ab,ti.) NOT ((polymorph* OR SNP) NOT (mutation* OR exome* OR delet* OR splic*)) NOT (exp animals/ NOT humans/) AND english.la. NOT (news OR congres* OR abstract* OR book* OR chapter* OR dissertation abstract*).pt.

Cochrane (RCTs) – 428 refs

((((obes* OR BMI OR (body NEXT/1 mass*) OR weight* OR overweight*) AND (LEPR OR leptinreceptor* OR ((leptin* OR LEP) NEAR/3 (receptor*)))) OR (((obes* OR BMI OR (body NEXT/1 mass*) OR weight* OR overweight*) NEAR/5 (gene OR genes OR genom* OR mutat* OR genet* OR monogen* OR nonsyndrom*))) AND (exome* OR sequencing* OR delet* OR mutat* OR variant* OR splic*)):ab,ti) AND ((clinical* OR case OR cases OR patient* OR cohort* OR male* OR female* OR man OR men OR woman OR women OR girl* OR boy* OR child*):ab,ti) NOT ((polymorph* OR SNP) NOT (mutation* OR exome* OR delet* OR splic*))

Web of Science – 3390 refs

TS=(((((obes* OR BMI OR body-mass* OR weight* OR overweight*) AND (LEPR OR leptinreceptor* OR ((leptin* OR LEP) NEAR/2 (receptor*)))) OR (((obes* OR BMI OR body-mass* OR weight* OR overweight*) NEAR/5 (gene OR genes OR genom* OR mutat* OR genet* OR monogen* OR nonsyndrom*))) AND (exome* OR sequencing* OR delet* OR mutat* OR variant* OR splic*))) AND ((clinical* OR case OR cases OR patient* OR cohort* OR male* OR female* OR man OR men OR
woman OR women OR girl* OR boy* OR child*)) NOT ((polymorph* OR SNP) NOT (mutation* OR exome* OR delet* OR splic*)) NOT ((animal* OR rat OR rats OR mouse OR mice OR murine OR dog OR dogs OR canine OR cat OR cats OR feline OR rabbit OR cow OR cows OR bovine OR rodent* OR sheep OR ovine OR pig OR swine OR porcine OR veterinar* OR chick* OR zebrafish* OR baboon* OR nonhuman* OR primate* OR cattle* OR goose OR geese OR duck OR macaque* OR avian* OR bird* OR fish*) NOT (human* OR patient* OR women OR woman OR men OR man))) AND DT=(Article OR Review) AND LA=(English)

Google Scholar – 200 refs (random-top-200)

obese|obesity LEPR|"leptin|LEP receptor"|leptinreceptor clinical -polymorphism|-polymorphisms|-SNP

NB: Studies describing novel heterozygous likely pathogenic variants in patients with obesity were not considered for inclusion as it remains unclear whether homozygosity or compound heterozygosity for these variants would have led to a clinical phenotype of LepR deficiency.

Supplementary Table S1. Overview of clinical characteristics of patients with LepR deficiency

Because this file is less informative in print due to its size and lay-out, the digital file can be accessed via: https://eje.bioscientifica.com/view/journals/eje/182/1/EJE-19-0678.xml?body=supplementaryMaterials-10421

Supplementary table S2 GnomAD allele frequencies

Source of mutation	Genomic position chr1 (gnomAD notation)	Aberration on protein level (gnomAD notation)	Allele Count (AC) Eur non-Finnish	Allele Number (AN) Eur non-Finnish	Allele frequency (AF) Eur non-Finnish	AC Finnish	AN Finnish	AF Finnish
gnomAD database pLoF	66036197	p.Thr29TyrfsTer6	1	113510	8,8098E-06	0	21590	0

gnomAD database pLoF	66036246	p.Tyr46Ter	1	113632	8,80034E-06	0	21630	0
gnomAD database pLoF	66036415	p.Leu101TyrfsTer15	2	15428	0,000129634	0	3476	0
gnomAD database pLoF	66038099	p.Tyr155IlefsTer13	0	111206	0	2	21466	9,31706E-05
gnomAD database pLoF	66058521	p.Met227AsnfsTer12	1	113228	8,83174E-06	0	21638	0
gnomAD database pLoF	66062229	p.Gln268Ter	1	15412	6,48845E-05	0	3468	0
gnomAD database pLoF	66064342	p.? (splicing defect c.850-1G>A)	1	113542	8,80731E-06	0	21628	0
gnomAD database pLoF	66067307	p.Tyr411LeufsTer4	1	113358	8,82161E-06	0	21620	0
gnomAD database pLoF	66067643	p.? (splicing defect c.1403+1_1403+2dupGT)	1	113592	8,80344E-06	0	21648	0
gnomAD database pLoF	66074585	p.? (splicing defect c.1752+1G>A)	3	128788	2,32941E-05	0	25116	0
gnomAD database pLoF	66075790	p.? (splicing defect c.1912+3_1912+15dupCTGCAGAGATTTT)	1	113744	8,79167E-06	0	21646	0
gnomAD database pLoF	66075910	p.Glu644LeufsTer6	0	128986	0	4	25116	0,000159261
gnomAD database pLoF	66075921	p.Trp646Ter	1	15426	6,48256E-05	0	3476	0
gnomAD database pLoF	66075946	p.Glu657GlyfsTer15	1	113316	8,82488E-06	0	21640	0
gnomAD database pLoF	66083646	p.? (splicing defect c.2213-1G>T)	1	15424	6,4834E-05	0	3456	0
gnomAD database pLoF	66083751	p.Glu773Ter	1	113450	8,81446E-06	0	21466	0
gnomAD database pLoF	66083777	p.Ile783SerfsTer37	1	113420	8,81679E-06	0	21336	0

gnomAD database pLoF	66087142	p.? (splicing defect c.2597+1G>T)	1	113524	8,80871E-06	0	21530	0
gnomAD database pLoF	66102123	p.Glu975Ter	1	15430	6,48088E-05	0	3476	0
gnomAD database pLoF	66102425	p.Tyr1078IlefsTer2	3	113010	2,65463E-05	0	21598	0

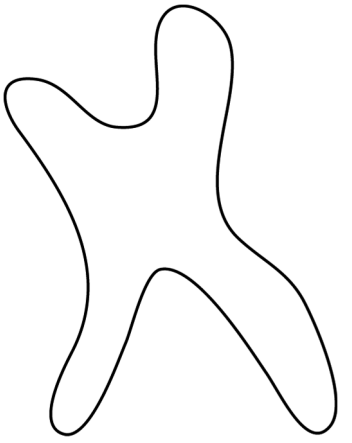
Source of mutation	**Variant in coding DNA**	**Aberration on protein level**	**Allele Count (AC) Eur non-Finnish**	**Allele Number (AN) Eur non-Finnish**	**Allele frequency (AF) Eur non-Finnish**	**AC Finnish**	**AN Finnish**	**AF Finnish**
Le Beyec *et al.*, 2019 (35)	N/A (start lost)	p.Met1*	1	113632	8,80034E-06	0	21648	0
Mazen *et al.*, 2011 (17)	c.946C>A	p.Pro316Thr	2	113412	1,76348E-05	0	21632	0
Hannema *et al.*, 2016 (25)	c.1604–8A>G	p.Lys536Serfs*34 and p.Val535Aspfs*3 (two transcripts)	1	112058	8,92395E-06	0	21578	0
Albuquerque *et al.*, 2014 (28); Farooqi *et al.*, 2007 (4); This publication	c.1835G>A	p.Arg612His	63	129162	0,00048776	1	25120	3,98089E-05
Farooqi *et al.*, 2007 (4)	N/A	p.Trp664Arg	6	112906	5,31416E-05	1	21630	4,62321E-05
Farooqi *et al.*, 2007 (4); Kohlsdorf *et al.*, 2018 (26); Kleinendorst *et al.*, 2018 (7); This publication	c.2051A>C	p.His684Pro	5	129146	3,87159E-05	0	25122	0
Huvenne *et al.*, 2015 (24)	c.2357T>C	p.Leu786Pro	1	113392	8,81896E-06	0	21206	0

gnomAD, genome aggregation database; chr, chromosome pLoF, predicted loss-of-function. The numbers in brackets after author name and publication year refer to the reference numbers in the article.

Part III

THE IMPORTANCE OF A DIAGNOSIS IN RARE GENETIC OBESITY DISORDERS

Chapter 6

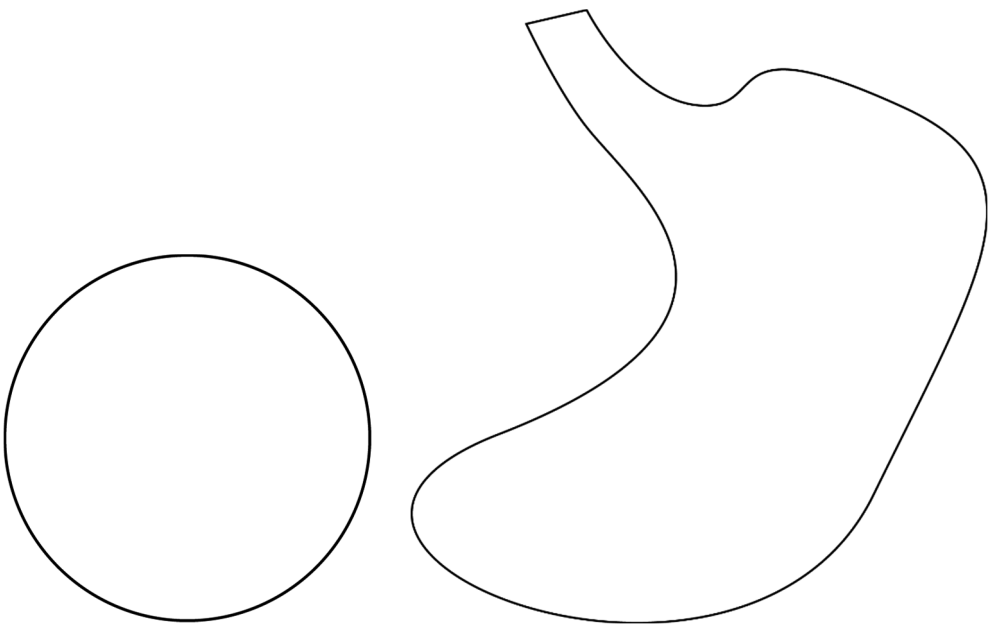


6.1

Young girl with severe early-onset obesity and hyperphagia

L. Kleinendorst, M.M. van Haelst, E.L.T. van den Akker

BMJ Case Reports 2017



ABSTRACT

Summary This case report of an infant with severe early-onset obesity illustrates the societal condemnation of persons with obesity. In addition, it underlines the importance of diagnosing rare forms of monogenic obesity, even if no drug treatment is available. Here, we describe a 2-year-old girl with severe progressive obesity from birth onwards due to insatiable hunger. Genetic studies eventually reveal that the girl has a monogenic form of obesity caused by two mutations in the *LEPR* gene. No drug treatment is available (as yet) for this disease. Parents describe the stigmatic remarks they have to deal with every day. Diagnosing this rare genetic disorder was very important for understanding that satiety regulation is a complex system, of which willpower is only a small portion. In these patients, reduction of obesity can be achieved, but a different approach to lifestyle intervention is needed.

Background

Obesity is a common problem that almost every physician or healthcare professional encounters. Genetic obesity however, is less known among clinicians. In these rare cases, genetic factors play a larger role than the behavioural and environmental factors that we usually associate with the causes of obesity. Many people still assume that obesity is just a matter of lacking the willpower to comply to a diet. However, satiety regulation is a much more complex system, and most non-syndromic monogenic obesity genes are involved in the brain's neuroendocrine satiety system. The patient described in this article is a striking example of the problems that occur when this satiety system is not functioning properly. Currently, we are not able to treat these diseases, except for leptin deficiency, but the first clinical trial with MC4R-agonists for patients with a specific type of monogenetic obesity (proopiomelanocortin deficiency) was recently performed with positive results.(1) By diagnosing the exact cause in genetic obesity, personalised treatment might be realised for all these different gene defects.

Case presentation

The girl was born premature at 34 weeks of gestation with birth weight of 2605 g (+1.9 SD for age) to non-consanguineous parents. Because of her preterm birth, she was admitted to the hospital and needed respiratory support for a couple of days. After 10 days, she was discharged.

A couple of weeks later, the girl had changed from a quiet neonate into an inconsolable baby. She cried day and night and could only be consoled with extra bottle feeding. At 11 weeks, she weighed more than 6 kg, and rolls of fat were appearing around her arms and legs. Her parents sought advice from the healthcare professionals of the community centre and were referred to a paediatrician.

Meanwhile, the parents tried harder and harder to follow the nutrition guidelines, with continuous crying and screaming of the girl as a result. Unfortunately, she did not lose weight at all, she gained weight at a more alarming rate than ever before. At the age of 6 months, the infant weighed almost 15 kg and she could not fit in her baby carriage.

The couple returned to the paediatrician with their daughter when she was 9 months old. The doctor was disturbed by the girl's weight and immediately referred her to an academic hospital.

Investigations

Hormonal disorders like hypothyroidism, hypocortisolism and hypercortisolism were excluded. Other laboratory tests showed that the girl's health status was already suffering from her obesity: hypercholesterolaemia and insulin resistance were detected. Her basal

energy expenditure, measured by indirect calorimetry, was 24% lower than normal for her weight (972 kcal, measured with indirect calorimetry using the Schofield equation).

Because of the combination of severe early-onset obesity and hyperphagia, we suspected the girl of a monogenic form of obesity. We tested her for the most common early-onset genetic obesity type, melanocortin-4 receptor deficiency, but no mutations were found in the *MC4R* gene.(2) Her leptin levels, measured in blood using a radioimmunoassay, were appropriately elevated due high fat mass, excluding leptin deficiency. The blood was not analysed for bioinactive leptin. There were no signs indicating a syndromic form of genetic obesity, for example, developmental delay, short stature, macrocephaly, dysmorphic signs or visual or hearing impairment.

The girl was admitted to the hospital for various diagnostic tests and the fine tuning of a low caloric diet. At that time, aged 1 year and 9 months, she was 88 cm tall (+1 SD), weighed 30 kg (+7.9 SD) and had a body mass index (BMI) of 38.7 kg/m^2 (+8.2 SD). Serious motor development limitation and genua vara were identified, caused by the extreme amount of fat tissue.

In the meantime, a diagnostic next-generation sequencing panel for genetic obesity became available. This test is aimed at the sequencing 52 obesity-related genes. With the use of the obesity gene panel, we could diagnose the girl with a monogenic form of obesity: leptin receptor deficiency. Two different (compound heterozygous) mutations in the *LEPR* gene were identified: c.1985T>C p.(Leu662Ser) and c.2168C>T p.(Ser723Phe), confirmed by Sanger sequencing. Sanger sequencing also showed that the parents are both carrier for one of the mutations. The variants are not previously found in other obese patients nor in the ExAC database (http://exac.broadinstitute.org/) of healthy controls. The *LEPR* mutations occur in highly conserved regions of the gene, suggesting an important role in the functioning of the receptor.

Differential diagnosis

Young children with early-onset obesity and hyperphagia may have an underlying genetic defect causing these problems. It is important to assess whether it is more likely a lifestyle related, a syndromic or non-syndromic type of obesity. The extreme early onset of the obesity is suggestive for a non-lifestyle caused obesity. Syndromal genetic obesity was not likely, because the infant showed no dysmorphic facial features or congenital anomalies, had a normal head circumference and there was no developmental delay. Therefore, monogenic obesity was suspected. Important types of monogenic obesity to diagnose are leptin and proopiomelanocortin (POMC) deficiency, since these can be effectively treated with leptin or setmelanotide injections, respectively. Leptin deficiency was excluded because of adequate elevated levels of leptin. POMC deficiency was excluded because of

the absence of hypocortisolism and red hair. At the moment it is not possible to differentiate very easily between the other types of monogenic obesity based on phenotype or clinical chemistry tests, so we used a multigene sequencing panel to establish the diagnosis of leptin receptor deficiency.

Treatment

No drug treatment is available as yet for patients with leptin receptor deficiency. However, it was a relief for the girl's parents to finally understand her problem and explain it to their family and friends. We tried to find supportive treatment for their daughter in various ways, referring for parental support for coping with the hyperphagic behaviour and the hurtful stigmatising comments made by strangers. We also referred the girl to the rehabilitation physician who designed adapted shoes and a custom-built stroller, as three regular strollers had already collapsed under her weight.

Outcome and follow-up

After the diagnosis was made, the girl's extreme weight slowly stabilised. At the age of 2 years (figure 2), her BMI was 38.7 kg/m^2 (+8.7 SD for her age group). In the first 4 months after the diagnosis her BMI lowered to 30 kg/m^2 (+6 SD), as seen in figure 1.

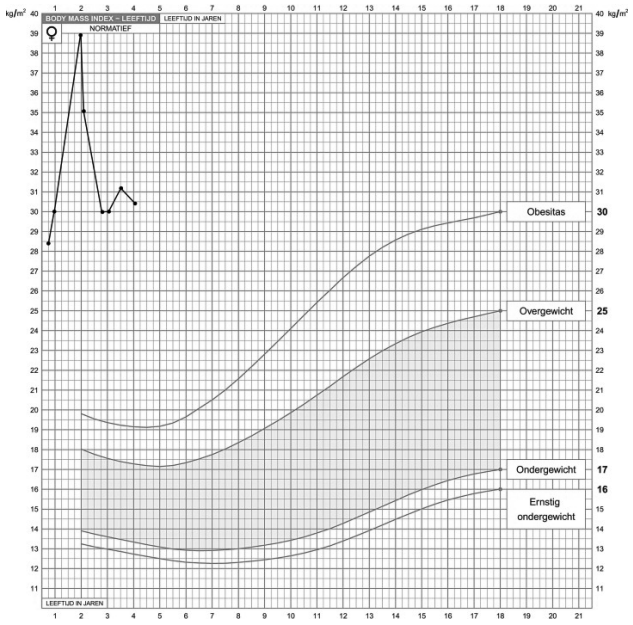


Figure 1. The patient's growth chart: body Mass index for age (0–4 years)

The identification of the *LEPR* mutations and thus the end of the diagnostic odyssey helped in the control of her weight, even without the availability a specific treatment for leptin receptor deficiency. To our knowledge there are no other examples of this observation published in literature, but we see this quite regularly in our genetic obesity clinics. Both the supportive treatment and the better understanding of the hyperphagic behaviour may enhance this effect.

The girl is now 4 years old and the fight against hunger remains, but her parents are doing the best they can to support their daughter. Diagnosing this rare genetic disorder has been of great importance even though drug treatment is not yet available for this genetic disorder.

Figure 2. The patient at the age of two

Discussion

Our patient was diagnosed with leptin receptor deficiency. This is a rare condition that causes early-onset obesity, mostly because of increased hunger and the accompanying overeating.(3) Leptin receptor deficiency is caused by homozygous or compound heterozygous mutations in the *LEPR* gene. Normally, the leptin receptor is activated by the hormone leptin, which is secreted by adipocytes. The amount of leptin in blood rises when adipocytes increase in size. Leptin binds to the leptin receptor causing various reactions in the hypothalamus that affect the energy balance by inhibiting appetite. Because the leptin receptor does not respond properly to leptin, it is not possible to treat these patients with leptin injections, as can be done for patients with a leptin deficiency.(3) Future studies are awaited to see if setmelanotide is effective in these patients. It is questionable if patients with homozygous leptin receptor mutations might be candidates for bariatric surgery taking into account the underlying defect. One case report from 2013 described a patient with successful weight loss and maintenance of the weight loss at 12 months after bariatric

surgery.(4) Another case report described a patient with a homozygous *LEPR* mutation that showed limited weight loss and weight regain a year after bariatric surgery.(4, 5)
Even though leptin receptor deficiency is rare, the 25 children reported in literature illustrate the complexity of the disturbed food intake and satiety regulation causing obesity.(6) The insatiability of LEPR-deficient children demonstrates the power of the brain's neuroendocrine satiety system. It is important that clinicians are aware of genetic causes of obesity and the new technologies available to test for the diseases. Since specific treatment options for monogenetic obesity are now being used in medical trials, the diagnosis can be of great importance for the patient. Moreover, a diagnosis helps in treatment strategy by using tailored lifestyle interventions and it can support the patients or their parents in coping with the social stigma of obesity. The hyperphagic behaviour associated with this disease is not a 'character flaw' after all but part of the disease.

Genetic testing is currently advised in the guideline of the Endocrine Society for obese patients with an onset before the age of 5, combined with clinical signs of a genetic obesity syndrome like hyperphagia or a family history that is suggestive of a genetic cause.(7) Because there is no distinct phenotype for every genetic defect in the leptin-melanocortin pathway, it may be the most effective to use a multigene panel or whole exome sequencing analysis for genetic testing in these patients. Even though the non-syndromic monogenic obesity disorders are rare, the yield of these tests may be higher in selected populations.

Patient's Perspective

The story of the patient's mother:

"I felt so insecure and I often worried that I was to blame for my daughter's obesity. Was I overfeeding her? Did I give her the wrong food? Was this all my fault? Every time the doorbell rang when I was not expecting visitors, I feared that child protection workers would take away my daughter under suspicion of abuse or neglect. After all, many people told me that I was a bad parent by letting her become so obese. I was also extremely worried about my daughter's health problems, I was so scared that her heart would stop beating, breaking down under the burden of her obese body. Every morning when my little girl slept longer than expected, I just could not enter her bedroom. In fear that I would find her not breathing. I started to avoid to take her outside to prevent the emotional burden of the remarks people made. Strangers asked if we were feeding my daughter "frying fat shakes'. It is a daily fight against both our daughter's hunger and the comments we get from strangers. After the diagnosis was finally made, we felt more capable to take on this battle.'

Learning points

- Satiety regulation is not simply a matter of willpower. In patients with monogenetic obesity, the hypothalamic neuroendocrine satiety system is affected, leading to hyperphagia and early-onset obesity.
- Diagnosing a genetic obesity disorder can be of great importance even though drug treatment is not (yet) available in most cases. A diagnosis helps in treatment strategy by using tailored lifestyle intervention and it helps the patient and parents in their fight against stigmatisation.
- Continuous research will hopefully further elucidate the underlying genetic pathways with ultimately personalised treatment (lifestyle interventions or medication) based on the genetic cause of this and other genetic obesity disorders.

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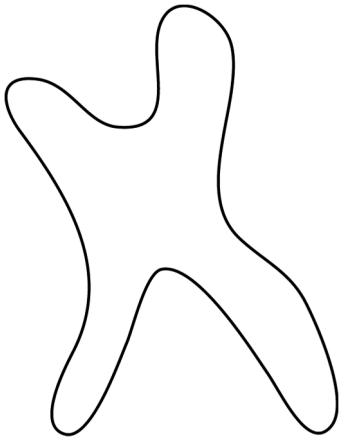

6.2

The role of obesity in the fatal outcome of Schaaf-Yang syndrome: Early onset morbid obesity in a patient with a *MAGEL2* mutation

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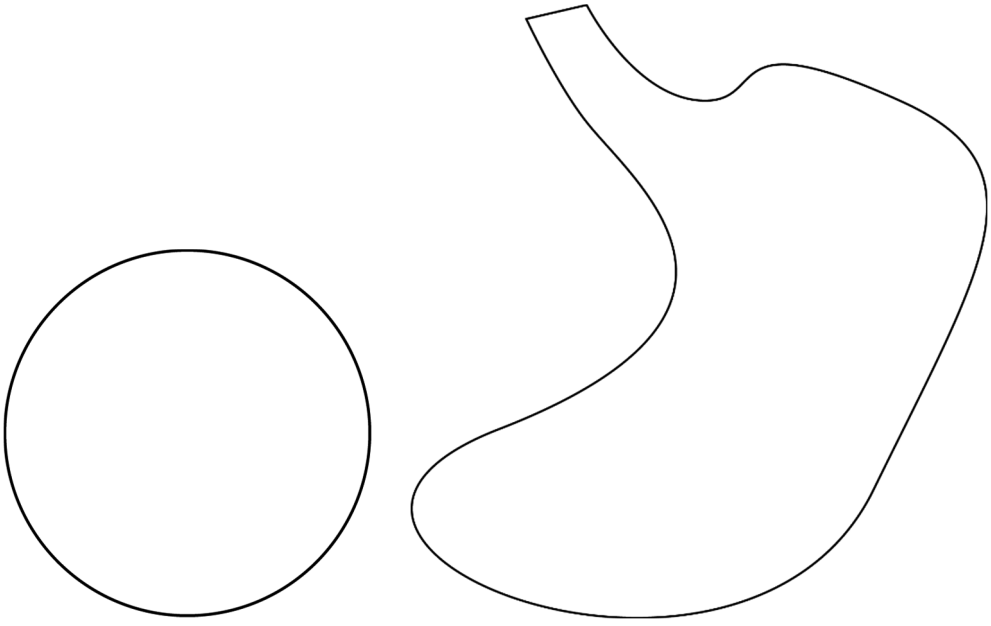

ABSTRACT

Schaaf–Yang syndrome (SYS) was recently identified as a genetic condition resembling Prader– Willi syndrome. It is caused by mutations on the paternal allele of the *MAGEL2* gene, a gene that has been mapped in the Prader–Willi critical region. Here, we present an infant with SYS who sadly died because of the combination of hypotonia, sleep apnea, and obesity. A heterozygous premature stop mutation in *MAGEL2* was identified in the patient. The main factors reported in the mortality of SYS are lethal arthrogryposis multiplex congenita, fetal akinesia, and pulmonary problems. Our clinical report indicates that obesity and its complications are an important additional factor in the mortality associated with SYS. Therefore, we advise to strictly monitor weight and intensively treat overweight and obesity in SYS.

Introduction

Schaaf–Yang syndrome (SYS) was recently identified as a genetic condition resembling Prader–Willi syndrome (PWS).(1) It is caused by mutations on the paternal allele of the *MAGEL2* gene, a gene that has been mapped in the Prader–Willi critical region.(2) The Prader–Willi phenotype in SYS manifests itself in neonatal hypotonia, feeding difficulties, and intellectual disability. Furthermore, patients with SYS typically have arthrogryposis and autism spectrum disorder. Most patients with SYS do not progress into the hyperphagic phase of PWS after the initial feeding problems. Obesity or excessive weight gain is therefore less seen in patients with SYS than in PWS.(3) Thus far, 45 patients with SYS have been reported in literature.(1, 3-12) Here, we report the fatal outcome of a toddler with SYS caused by complications secondary to her morbid obesity which is a known feature of the syndrome. This case clearly illustrates the fatal combination of hypotonia, pulmonary problems, apnea, and obesity in SYS.

Methods

Clinical report

The proband was the first child of nonconsanguineous Spanish parents. She was born at term by caesarean section because of breech position. Prenatal ultrasounds showed no abnormalities apart from decreased fetal movement. The Apgar scores were 6, 8, and 10 after 1, 5, and 10 min, respectively. Birth weight was 3700 g (p75), length was 49 cm (p30), and the occipitofrontal circumference was 36 cm (p90) (Table S1, Supporting Information). Immediately postnatally, scarce spontaneous movements, hypotonia, and a laryngeal stridor were noticed. Apart from arthrogryposis (of hand, shoulders, hips, and knees), several dysmorphic features were observed: a square-shaped face, upslanted palpebral fissures, limited opening of the mouth, a horizontal chin crease, and a short neck. Because of initial feeding problems, she received nasogastric tube feeding. After 1 week, the nasogastric tube was removed and she could be discharged from the hospital.

At the age of 5 months, there was improvement of the contractures, especially in the hands. Her hypotonia and stridor persisted and she showed continuous protruding movements of the lips. Her hands and feet were edematous. At the age of 10 months, she could sit without support; however, severe muscular hypotonia was still present. She developed severe truncal obesity, even though she was on a balanced diet appropriate for her age. According to her parents, there was no clear increase in intake. At the age of 20 months, it was clear that she had a severe delay in her development; she could not speak and could only stand up with support. Her body mass index (BMI) at that time was 8 *SD* above normal for her age and gender.

At the age of 22 months, she had to be hospitalized for bronchopneumonia after a choking episode. She required respiratory support with noninvasive ventilation for 8 days. She

recovered without complications. During this hospital admission, no apnea episodes or oxygen desaturations were observed. Biochemical studies showed normal results: glucose 4.8 mmol/L, glycosylated hemoglobin 4.7%, thyroid-stimulating hormone (TSH) 1.75 mIU/L, and normal cholesterol and triglyceride levels.

At the age of 23 months, her parents found her lifeless in her bed. There had been no episode of illness and no chocking episodes were reported in the days prior to her death. In the postmortem study, no abnormalities were observed that could explain the cause of her death. The pathologist noted a disproportionate increase in fat throughout the subcutaneous cellular tissue (Figure 1). Her BMI at that time was 34.5 kg/m^2, 13 *SD* above the mean. The conclusion of the medical team was that the patient most likely died of sleep apnea caused by her extreme obesity. As polysomnography was not performed, it remains unclear whether the apnea was central, obstructive, or a combination of these.

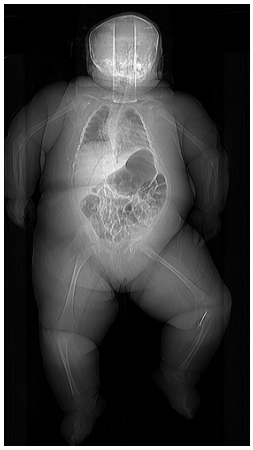

Figure 1. Postmortem X-ray image of the patient showing the extreme amount of adipose tissue

Molecular investigation

Molecular analysis was performed at the Hospital Universitario y Politécnico La Fe. Genomic DNA was extracted from blood leukocytes. An array-comparative genomic hybridization (CGH) showed no copy number variations. DNA methylation analysis showed normal imprinting within the Prader–Willi critical region. Myotonic dystrophy Type 1 was also excluded; there were no abnormalities in the CTG repeats of the polymorphic region at the 3' end of the *DMPK* gene. Sanger sequencing was performed and analysis showed a heterozygous premature stop mutation, NM_019066.4: c.1850G > A (p.Trp617*) in the *MAGEL2* gene. Determination of the parental origin of the *MAGEL2* mutation was performed according to Schaaf et al. 2013 and showed that the variant was located on the

paternal expressed allele, confirming the clinical diagnosis of SYS. Segregation analysis of the parents' DNA samples showed no abnormalities, indicating that the mutation had arisen de novo in the proband, although germline mosaicism cannot be excluded.

Genetic analyses of the patient were performed during the mother's second pregnancy. The parental results provided reassurance for the parents regarding the low recurrence risk of SYS in this case. In the meantime, a healthy baby was born.

Discussion

Our case report clearly shows that complications of severe obesity can be an important risk factor in the mortality associated with SYS and that the development of obesity should be intensively monitored and treated in these patients. A fatal outcome in patients with SYS was reported 10 times in literature, mostly because of lethal arthrogryposis multiplex congenital or fetal akinesia.(3, 9) There are four previously reported patients in literature with a *MAGEL2* mutation who died in early childhood like our patient. A 9-month-old male child's suspected cause of death was apnea.(3) A Chinese girl passed away at the age of 11 months due to cardiovascular failure after a hypoglycemic episode. Another Chinese child, a male child aged 2 months passed away as well. He suffered from dyspnea without an obvious cause.(11) Unfortunately, both authors did not report whether the patients were obese. A girl who was described in 2017 died after publication of the article, as noted in the acknowledgments of a recent publication.(3, 8) She passed away unexpectedly at the age of eight. The cause of death was not mentioned, but she was not obese.

Fountain et al. noted that further investigation of SYS's associated mortality is necessary.(3) Later, Enya et al. suggested that pulmonary problems caused by the *MAGEL2* effect in the lung and muscular dysfunction can lead to early death in Schaaf–Yang patients.(5) We here suggest that early onset obesity is an additional risk factor for possible fatal outcome in SYS. Childhood obesity is a worldwide problem that has both short- and long-term negative health effects on many different organ systems.(13) In PWS, obesity is the major cause of mortality.(2) An important factor in the obesity-associated mortality is sleep apnea. The mechanic obstruction of the pharyngeal airway and reduced lung volume because of truncal adipose tissue seem to play a key role in the causal mechanism between obesity and sleep apnea.(14) One can imagine that movement of the chest was severely limited in our patient because of the large amount of body fat (Figure 1). Sleep apnea is also observed in SYS patients without obesity (Table 1), therefore we think the obesity aggravates the mortality risk in SYS.

Another, more long-term, negative health effect of obesity is the metabolic syndrome. A recent study of the hormonal and metabolic phenotype of nine children and adolescents

with SYS showed that two of the patients were prediabetic and one patient was diabetic.(8) This could be another reason for strict monitoring of weight in patients with SYS.

Approximately 42% of the patients with SYS reported in literature had excessive weight gain or obesity (Table 1). It is unclear why the obesity phenotype is present in only half of the patients with SYS. This relative low prevalence could suggest that this phenotype is not attributable to loss of *MAGEL2* expression alone. However, this needs to be investigated further. It is hypothesized that the weight gain is mainly caused by dysfunction of hypothalamic pathways that control both appetite and energy expenditure.(15) This could explain why our patient became obese even when there was no evident hyperphagia reported by the parents. McCarthy et al. showed that children with SYS have elevated fasting ghrelin levels, comparable to patients with PWS, even though none of their reported patients are hyperphagic.(8) Therefore, the explanation that hyperphagia in PWS is caused by elevated ghrelin levels might be more complicated than initially suggested. Future studies are needed to define the exact cause and effects of the high ghrelin in PWS and SYS. The resting energy expenditure of SYS patients might be low because of the decreased physical activity similarly to PWS caused by hypotonia and a low lean body mass.(2)

In conclusion, the fatal outcome in children with SYS can be influenced by the syndrome-associated pulmonary problems, hypotonia, apnea, and obesity. All these factors can have additionally total lethal effects. Therefore, we advise strict monitoring of weight in all Schaaf–Yang patients from the neonatal period onward (our patient became obese at the age of 10 months) and to refer the patient for treatment as soon as severe hyperphagia or overweight appears.

Table 1. Patient characteristics regarding the possible lethality of SYS of our patient and 34 published patients

Patient Charact-eristics	Schaaf 2013	Soden 2014	Mejlac-howicz 2015	Fountain 2017	Urreizti 2017	Enya 2018	Matusz-weska 2018	McCarthy 2018*	Jobling 2018	Tong 2018	Bayat 2018	This report	Summary	Summary
	(n = 4)	(n = 2)	(n = 4)	(n = 18)	(n=1)	(n=3)	(n=2)	(n=2)	(n=6)	(n=2)	(n=1)	(n=1)	(n =46)	(%)
Excessive weight gain or obesity	3/4	1/2**	NA	5/13	0/1	NR	NR	0/2	NR****	NR	0/1	1/1	10/24	**41.7%**
Hypotonia	2/4	NR	NA	13/13	1/1	3/3	2/2	NR	6/6	2/2	1/1	1/1	31/33	**93.9%**
Apnea	2/4	1/2	NA	9/13	1/1	NR	2/2	NR	3/6	1/2	NR	1/1	20/31	**64.5%**
Pneumonia	NR	NR	NA	NR	NR	1/3	NR	NR	1/6	0/2	NR	1/1	3/12	**25%**
Lethal arthrogryposis multiplex congenita or fetal akinesia	0/4	0/2	4/4	2/18	0/1	0/3	0/2	0/2	0/6	0/2	0/1	0/1	6/46	**13%**
Fatal outcome (reported in article)	0/4	0/2	4/4	4/18***	0/1	0/3	0/2	0/2	0/6	2/2	0/1	1/1	11/46	**23.9%**

NA: Not Applicable. NR: Not Reported. * McCarthy, 2018 presents 9 patients of which 7 patients were reported previously in Soden, 2014 and Fountain, 2017. Therefore, we only include the 2 new patients in this table. **Obesity was not reported in any of the patients in Soden, 2014, but one of them was later described as obese in McCarthy, 2018. *** One of the patients described in Fountain, 2014 passed away after publication of the article, described in McCarthy, 2018. ****Jobling, 2018 described increased subcutaneous fat in 3/6 patients.

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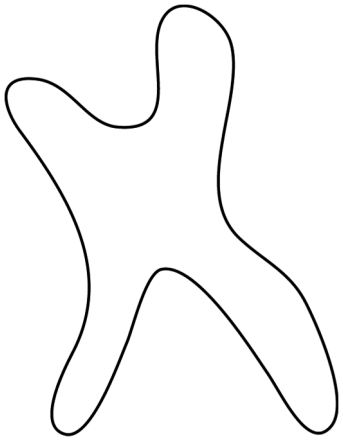
SUPPLEMENTARY APPENDIX

Table S1. Anthropometric measurements of the patient at hospital visits

Age ***months***	**Weight** ***kg***	**Weight percentile (SD)**	**Length** ***cm***	**Length percentile**	**BMI** ***kg/m²***	**BMI SD**	**Occipitofrontal circumference** ***cm***	**OFC percentile**
0	3.7	75	49	30	15.4	-	36	90
10	12.7	>99 (+3)	73	30	23.8	+4	45	75
19	19.8	>99 (+6.4)	81	30	30.2	+9	-	-
23	25	>99 (+9.2)	85	30	34.5	+13	49.5	70



6.3

Genetic analysis in the bariatric clinic; impact of a *PTEN* gene mutation

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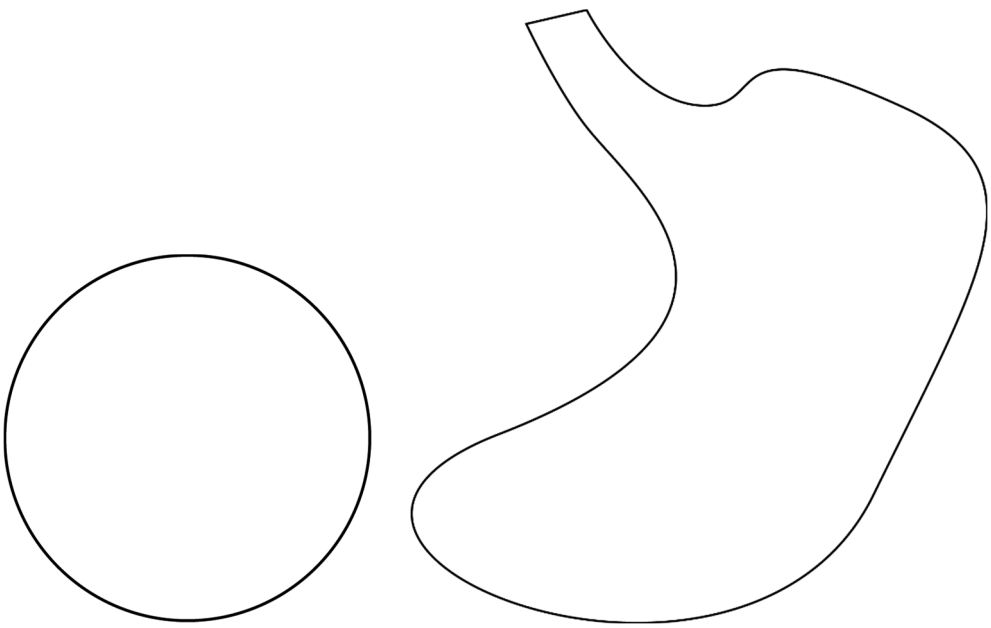

ABSTRACT

Background Pathogenic *PTEN* gene mutations are known to cause PTEN tumor hamartoma syndrome. Recent studies also suggest a role for *PTEN* mutations in the pathogenesis of obesity. No *PTEN* mutations have been reported among bariatric surgery patients and obesity treatment results are unknown. Since preventive screening for associated tumors is offered to patients with molecular proven PTEN hamartoma tumor syndrome, recognition of this condition in the bariatric surgery clinic is important.

Method We present a patient with morbid obesity who carries a known pathogenic *PTEN* mutation, identified at the bariatric surgery clinic using an obesity gene panel consisting of 52 obesity–associated genes. We analyzed the weight loss response during the first 3 years after Sleeve Gastrectomy.

Results At 1, 2 and 3 years after surgery, the patient achieved a Total Body Weight Loss of 39.4%, 48.8% and 44.9%, respectively. This corresponds to the results of a control group of 18 female patients with normal genetic test results.

Conclusion Our patient illustrates the importance of recognizing this serious genetic condition for which preventive cancer screening options are available. The positive weight loss results after Sleeve Gastrectomy suggest that this could be a successful treatment option for obesity patients with *PTEN* mutations.

Introduction

Bariatric surgery is an effective treatment option for obesity in the majority of patients.(1, 2) Besides following the criteria of the International Federation for the Surgery of Obesity and Metabolic Disorders, it is important to securely determine the obesity causing factors, to be able to select patients who are expected to benefit the most of weight loss surgery.(3) Multiple lifestyle- or endocrine/hormonal- factors, but also a genetic cause of obesity could be of great importance for the onset of obesity. Unfortunately, sufficient knowledge about the role of underlying genetic factors and the effect of bariatric surgery in patients with genetic obesity is still lacking. We here describe a patient with a mutation in the phosphatase and tensin homologue (*PTEN*) gene, a tumor suppressor gene with a regulatory role in the cell proliferation process. Patients with PTEN hamartoma tumor syndrome (PTEN HTS) usually present with mucocutaneous lesions (90%–100%), thyroid abnormalities (50%–67%), macrocephaly (38%) or genito–urinary abnormalities (44%) in combination with a family history of different types of cancers.(4, 5) Less often diagnosis can be made in children with a combination of macrocephaly and/or mild intellectual deficit. Recent studies have also suggested a role for *PTEN* mutations in the pathogenesis of obesity.(6) As far as we are aware, this is the first report of an obese patient with a *PTEN* mutation who successfully underwent bariatric surgery.

Case presentation

The index patient was a 34-year old female referred to the bariatric clinic by the general practitioner on her own request to treat her morbid obesity. She was born with a normal birth weight but large head circumference for which she never had a diagnostic analysis. At the age of five, her body weight was already significantly higher compared to her peers. No specific life events could explain her obesity. Cognitive development was normal and she followed normal education. She underwent treatment for recurrent nasal polyps. Her mother also had a large head size and suffered from morbid obesity as well. She was diagnosed with thyroid cancer and died from a pulmonary embolism after placement of an Adjustable Gastric Band. A maternal aunt was diagnosed with breast cancer before the age of 50 and the maternal grandmother died from breast cancer at young age. The younger sister of the index patient was overweight and was reported to also have a large head size (Figure 1).

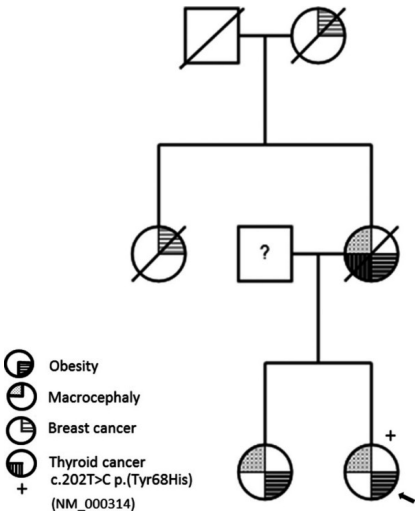

Figure 1. Pedigree

Since childhood, the index patient followed several different coaching programs to change her eating behavior and exercise pattern to induce weight loss. She lost weight several times but was never able to maintain her weight loss. At the time of the intake procedure at the bariatric clinic, her height was 1.69 m (*SD* −0.2) and weight 164 kg (*SD* +6.8), resulting in a Body Mass Index (BMI) of 57.6 kg/m^2 and a predominant abdominal obesity. Head size was not measured at that time since this is not part of bariatric screening procedures. Biochemical analysis of the blood revealed no abnormalities, and excluded endocrine hormonal disorders such as hypothyroidism. The fasting glucose level was 5.9 mM.

The combination of early onset morbid obesity resulted in suspicion of a genetic cause of her obesity. She was offered diagnostic genetic analysis of 52 obesity–associated genes to identify a possible underlying genetic obesity cause.

The patient was eligible for bariatric surgery and underwent a sleeve gastrectomy without complications (performed in 2014 using a standardized fashion). At 1, 2 and 3 years after surgery, she achieved a percentage Total Body Weight Loss of 39.4, 48.8 and 44.9, respectively. This resulted in a current BMI of 30.1 kg/m^2. This was within the range of the results which were observed in a control group of 18 female patients, with a negative obesity genetic test result. These female patients were matched for age and BMI and achieved a percentage Total Body Weight Loss (TBWL) of 30.3 after 1 year, 31 after 2 years and 30 after 3 years of follow-up.

A few months after surgery, the result of the obesity gene panel analysis was returned and showed heterozygosity for a known pathogenic mutation in the *PTEN* gene (NM_000314.4): c.202T>C p.(Tyr68His). This mutation has been described previously in patients with PTEN Hamartoma Tumor Syndrome (PTEN HTS).(7) No other pathogenic mutations were shown in the remaining 50 obesity–associated genes (Table 1). At the genetic clinic, a head circumference of 63 cm (+5*SD*) and pedigree analysis (family history of multiple tumors) further supported the molecular diagnosis of PTEN HTS.

According to the PTEN HTS guidelines, patients with pathogenic *PTEN* mutations are advised to visit the outpatient clinic for familial tumors, for lifelong surveillance of tumors that are associated with the *PTEN* mutations.(4, 5) Our patient underwent additional biochemical laboratory- and ultrasound screening to exclude thyroid gland carcinoma. Besides a few benign nodules on the ultrasound, no abnormalities could be determined. A yearly follow-up ultrasound of her thyroid gland and yearly serum thyroid stimulating hormone analysis was advised. Screening for breast, endometrium and colorectal cancer, also revealed no anomalies.

Table 1. Obesity gene panel 2014

ALMS1	*BBS12*	*IRS4*	*MKKS*	*PCSK1*	*TBX3*
ARL6	*BDNF*	*KIDINS220*	*MKRN3*	*PHF6*	*THRB*
BBS1	*CCDC28D*	*LEP*	*MKS1*	*POMC*	*TMEM67*
BBS2	*CEP290*	*LEPR*	*MRAP2*	*PRKAR1A*	*TRIM32*
BBS4	*CRHR2*	*LZTFL1*	*NDN*	*PTEN*	*TTC8*
BBS5	*FLOT1*	*MAGEL2*	*NTRK2*	*SIM1*	*TUB*
BBS7	*G6PC*	*MC3R*	*PAX6*	*SNRPD2*	*WDPCP*
BBS9	*IRS1*	*MC4R*	*PTHB1*	*SNRPN*	
BBS10	*IRS2*	*MCHR1*	*PCK1*	*SPG11*	

Custom Agilent SureSelect target enrichment assay followed by massive parallel sequencing on SOLiD5500XL sequencer: analysis of protein coding and flanking intronic sequences of 52 obesity and obesity comorbidity associated genes.

Discussion

Although obesity is suggested to be a multifactorial condition, mostly caused by our changing obesogenic environment, an underlying genetic defect has been reported in approximately 2%–15% of morbidly obese patients.(8, 9) Mutations in the melanocortine-4 receptor (*MC4R*) gene are the most common cause of monogenic obesity, with a prevalence of 0.5%–5.8%, with the highest values expected in cohorts with early onset obesity.(10)

Since genetic obesity diagnoses are often difficult to establish in obese adults, it is expected that part of the patients who undergo a bariatric surgical treatment might have an underlying genetic cause of obesity. Implementation of next generation sequencing analysis in daily clinical obesity care facilitates the identification of genetic causes of obesity. Because of the early onset morbid obesity, a genetic cause of obesity was suspected in our patient. There were no contra-indications to perform bariatric surgery, since this was the only remaining treatment option to achieve durable weight loss.

Monogenic obesity conditions are most often detected during childhood when patients present a combination of congenital malformations, dysmorphic features and/or intellectual problems. The combination of morbid obesity and macrocephaly could also suggest a 16p11.2 deletion syndrome. The prevalence of this genetic condition in the general population is estimated at 3 in 10,000. It is mostly associated with autism spectrum disorder and learning- and speech problems, but it is also a 43-fold increased risk for morbid obesity.(11, 12) The family history of breast- and thyroid cancer and the normal development in our patient made this diagnosis less likely.

The association of obesity and *PTEN* mutations is not well understood. Garcia-Cao et al. and Ortega-Molina et al. showed that overexpression of *PTEN* in mice leads to reduced body weight and size, combined with hyperphagia.(13, 14) This suggested a poor energy storage capacity, which was confirmed by calorimetric measurements showing increased energy expenditure and oxygen consumption in these mice.(15) This was further supported by the finding of elevated activity of brown adipose tissue in *PTEN* overexpressed mice.(13)

In humans, Pal et al. showed a strong association between *PTEN* loss of function mutations resulting in expected haploinsufficiency and the presence of obesity.(16) Fifteen *PTEN* mutation carriers had a mean BMI of 32 kg/m^2 (range 23–42) compared with 26 kg/m^2 in fifteen matched controls (range 15–48), showing that the *PTEN* affected patients were clinically significantly overweight (p = 0.001). Data from bone densitometry, did however show no significant differences in lean mass, bone mineral content or total fat between the patients with a *PTEN* mutation and controls. The authors state that the higher BMI in patients with *PTEN* mutation could be attributable to an increase in adipose tissue. Their presented data do however not yet support this conclusion, since there was no significant difference in skinfold thickness between the patients and the controls.(14, 16, 17) So the exact role of *PTEN* associated obesity still remains unclear and further research is needed to determine the mechanism behind the reported higher BMI in patients with *PTEN* mutation.

PTEN HTS is rare and difficult to diagnose if not familiar to the clinician. Especially since the prevalence in selected groups, such as obese patients in the bariatric clinic, is not known.

The results after sleeve gastrectomy were good in our patient and comparable with a control group of matched patients. However, no definitive conclusion can be drawn from this positive result since this is the first report of a patient with a *PTEN* mutation who underwent bariatric surgery. More research is needed to determine the best treatment possibilities for these patients.

Although weight loss reduces the risk of cancer development in the general population, timely identification of *PTEN* mutations in early onset obesity patients can further result in a major health benefit. This is also of great importance for other family members who are at risk of sharing the same genetic defect. Since the mother, the maternal aunt and maternal grandmother were reported to have clinical features fitting with a diagnosis of PTEN HTS, it is highly suggestive that our patient inherited a familial *PTEN* mutation. The sister of our patient was referred to the genetic department as well. Unfortunately we do not have any further information on her.

In conclusion, we here report a case with morbid obesity associated with a pathogenic *PTEN* mutation. The sleeve gastrectomy in this case resulted in successful weight loss in the first 3 years after surgery, but more cases with a *PTEN* mutation who underwent bariatric surgery need to be reported. Long term follow-up results and further clarification of *PTEN* mutations in the pathogenesis of obesity, might lead to further personalized treatment options.

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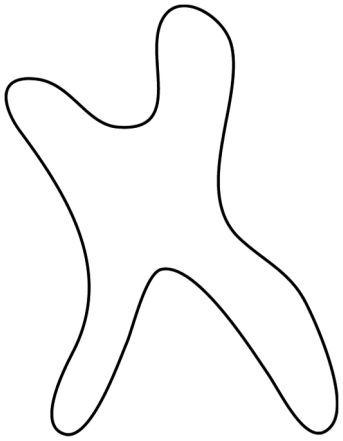
We are grateful to the patient for her cooperation and permission regarding the publication. Written informed consent for publishing the anonymous data was given.



6.4

Second case of Bardet-Biedl syndrome caused by biallelic variants in *IFT74*

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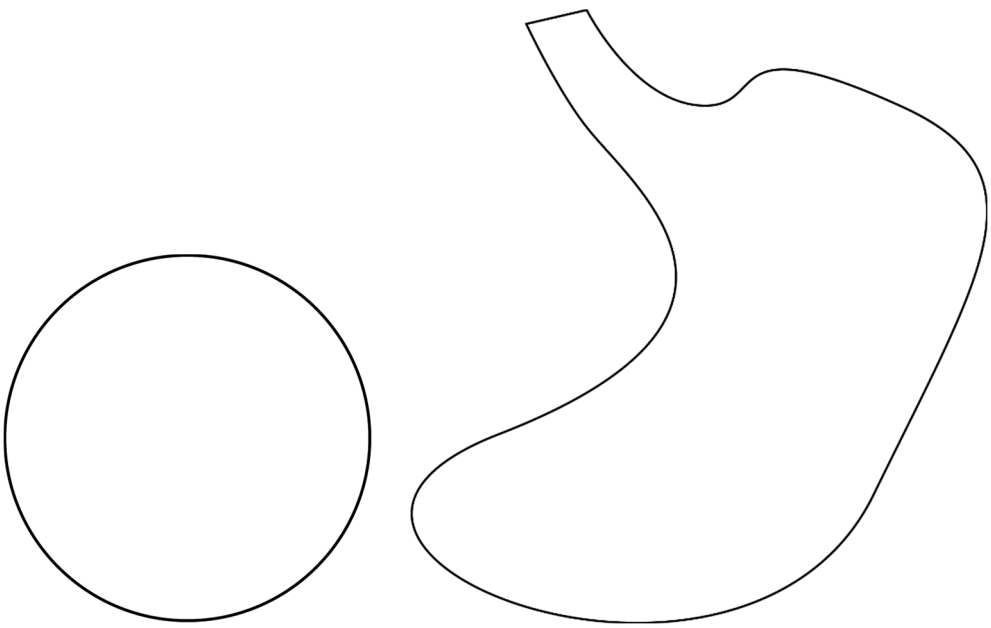

ABSTRACT

Bardet–Biedl syndrome (BBS) is a rare autosomal recessive disorder of the cilia, often resulting in a phenotype of obesity, rod-cone dystrophy, a variable degree of intellectual disability, polydactyly, renal problems, and/or hypogonadism in males or genital abnormalities in females. We here report the case of an 11-year-old girl who presented with postaxial polydactyly, retinal dystrophy, and childhood obesity, suggesting Bardet–Biedl syndrome. She had no renal problems, developmental delay, or intellectual disability. Genetic testing revealed compound heterozygous variants in the *IFT74* gene (c.371_372del p.Gln124Argfs*9 and c.16850–1G>T p.?). We here report the second patient with Bardet–Biedl syndrome due to biallelic *IFT74* variants. Both patients have obesity, polydactyly, retinal dystrophy, and no renal abnormalities. The present case however, has normal intellect, whereas the other patient has intellectual disability. We hereby confirm *IFT74* as a BBS gene and encourage diagnostic genetic testing laboratories to add *IFT74* to their BBS gene panels.

Introduction

Bardet–Biedl syndrome (BBS) is a rare autosomal recessive ciliopathy characterized by polydactyly, rod-cone dystrophy, renal abnormalities, obesity, and intellectual disability.(1) Currently, over 20 genes are associated with BBS.(2) BBS is most often caused by germline variants affecting function in *BBS1* and *BBS10*.(1)Some recently discovered BBS genes are members of the intraflagellar transport machinery (IFT). IFT is a bidirectional mechanism involved in the protein motility within the cilia and is important for both ciliogenesis and maintenance of the cilia.(3) There are three IFT genes associated with BBS: *IFT27, IFT74*, and *IFT172*.(2, 4) All three genes encode for parts of the IFT-B complex which is needed in the anterograde transport of ciliary proteins, whereas the protein complex IFT-A is required for the retrograde transport.(3). Most biallelic pathogenic variants in IFT genes are associated with skeletal ciliopathies such as short-rib thoracic dysplasia (OMIM # 617102, # 611263, # 617866) and cranioectodermal dysplasia (OMIM # 218330). Two of the BBS-associated IFT genes, *IFT74* and *IFT172*, are currently called "*BBS20"* in literature, which leads to confusion regarding which gene is in fact the BBS20-gene.(4-6) We would suggest using *IFT74* and *IFT172* only and discard *BBS20* for the sake of clarity. Biallelic pathogenic *IFT74* variants are associated with BBS20 in OMIM (OMIM # 617119) and have thus far only been reported in a single case.(5) We here report the second patient, confirming *IFT74* as one of the genes causing BBS when disrupted.

Subjects and methods

Case report

The proband is the second child of nonconsanguineous Dutch parents. She was born after an uncomplicated pregnancy of 41 weeks by cesarean section. Prenatal ultrasounds showed no anomalies. Birth weight was 4710 g (>2 SD for gestational age). Immediately after birth, postaxial polydactyly of the feet was noticed. There was a hemangioma at the left side of the jaw and earlobe, which later completely involuted. There were no craniofacial dysmorphisms. She had a large occipitofrontal circumference (OFC) of 40.8 cm at age one month (+3.3 SD). The additional toes were removed at age 11 months. She attained age appropriate milestones in all developmental sectors except for speech, speaking only five words at the age of 2 years but developing normal speech later in childhood. Because of her learning skills, she could skip a class in elementary school and will follow the highest level of secondary education in The Netherlands which grants access to university. Suboptimal vision was first noticed at the age of 5 years and ophthalmological examination showed macular hypopigmentation and a granular appearance. Currently, there is reduced central vision. Peripheral vision is intact. Ultrasound examination of the kidneys at age 1 year and age 8 years revealed no abnormalities of the kidneys. Age at menarche was 10 years and 11 months. No signs of genital abnormalities were noticed during physical examination and on ultrasound examination.

The proband presented at Obesity Center CGG in Rotterdam, The Netherlands at age 8 years and 10 months. She was referred for in-depth analysis of her obesity and to find a possible cause for her phenotype. Weight gain and hyperphagia started at the age of 4 years. Her eating pattern was normal and she had low-normal amounts of physical activity. At that time, height was 145.5 cm (+1.5 SD for age and sex); weight 52 kg; BMI 24.6 kg/m2 (+3 SD). She still had macrocephaly with an OFC of 58.4 cm (+3.8 SD). Indirect calorimetry revealed a 14% lower resting energy expenditure than predicted. Laboratory measurements regarding comorbidities of obesity, including standard oral glucose tolerance test, showed no signs of hepatic steatosis, and no dyslipidemia or impaired glucose tolerance. At her most recent visit at age 11 years and 2 months, her height was 162.7 cm (+1.8 SD), BMI was 26.8 kg/m^2 (+2.9 SD), and OFC of 58.2 cm (+2.9 SD). Her urine albumin/creatinine ratio was normal.

Family history

There are no other family members with polydactyly or retinodystrophy, nor are there family members with other signs of BBS that are not present in the proband. The OFC of the father was +2 SD above average for age and sex, the OFC of the mother was at 0 SD.

Genetic analysis

Prior to referral to our outpatient clinic, gene panel analysis for eye diseases and ciliopathies performed in 2014 could not identify a cause of the patient's phenotype. We performed whole-exome sequencing (WES). WES libraries were prepared using SeqCap EZ MedExome (Roche Sequencing, Pleasanton, CA) and sequenced on a HiSeq2500 platform. Using literature research, a selection of BBS and obesity associated genes was generated (Supplementary data). Variants found by WES were screened against the list of selected genes to detect known and novel causes of genetic causes of obesity and/or BBS. Variant classification was performed according to the recommendations of the American College of Medical Genetics and Genomics.(7) The identified variants were submitted to the Leiden Open Variation Database (LOVD). Sequential segregation analysis of detected variants in the patient's parent was performed using Sanger sequencing.

Results

Two heterozygous variants in *IFT74* (NC_000009.12, NM_ 025103. 3)*:* c. [371_372del] ;[1685–1G>T] p.[(Gln124Argfs*9)]; [p.?] were identified. The first variant is a novel deletion of two nucleotides causing a frameshift and premature stop, which would likely lead to nonsense-mediated decay. The second variant is an intronic variant in the splice consensus sequence, which has already been described in the other *IFT74* patient (ClinVar RCV000240867.2).(5) Splice prediction software predicts a complete loss of this splice donor site. Both variants were classified as probably pathogenic. Sanger sequencing showed that the variants were inherited from the parents, confirming that the variants are indeed

biallelic. Both the variants and the phenotype are submitted to LOVD (https://databases.lovd.nl/shared/genes/IFT74). The variant IDs are #0000604191 and #0000604192, respectively. The individual ID is #00269286 and the phenotype ID is #0000210664.

Discussion

This is the second report of *IFT74* variants causing a BBS, validating *IFT74* as a *BBS* gene. So far only one previous case of BBS caused by biallelic *IFT74* variants has been published by Lindstrand et al. in 2016.(5) This patient was a 36-year-old male with retinitis pigmentosa, microcephaly, obesity, polydactyly, hypogonadism, and no renal abnormalities. Lindstrand et al. report that their patient had intellectual disability but without developmental delay (sic). This phenotype differs from the phenotype in our patient (Table 1). Interestingly, our patient had macrocephaly, whereas the previously reported patient had microcephaly. Macrocephaly is more frequently observed in BBS cases than microcephaly.(8)The macrocephaly could also be familial in our case, since the father has an OFC of +2SD. Moreover, the phenotype difference between the two patients is especially important regarding the normal intelligence in our patient and intellectual disability in the Lindstrand patient. We want to emphasize this difference because of its importance for prenatal diagnostics and genetic counseling. Around 60% of BBS patients have learning difficulties, which are usually mild to moderate.(9) The intellectual phenotype of *IFT74*-associated BBS now ranges from intellectual disability to normal intellectual capacity. Both cases did not have renal anomalies. Since renal problems are observed in 53–82% of BBS cases, more cases need to be described to find out if absence of renal problems in our cases are coincidental or if the renal phenotype is less severe in *IFT74*-associated BBS cases.(1) Future studies and reports of BBS patients with *IFT74* variants are needed to gain insight in the complete clinical spectrum and the causes of the phenotype differences. The previously reported case had the same splice variant as our patient (c.168501G>T), but the second variant was different. There was a deletion of ∼20 kb encompassing exons 14–19 of the long transcript of *IFT74*. The deletion does not impair the function of the short isoform. The splice variant that occurs in both patients also affects the long isoform. Lindstrand et al. hypothesize that their proband is hypomorphic for IFT74 function. The second variant found in our patient (c.371_372del p.(Gln124Argfs*9)) causes a premature stop which would affect both isoforms. With only two known patients, it is difficult to predict whether there could indeed be a genotype–phenotype correlation.

With regards to phenotype differences, it remains to be investigated whether *IFT74* variants are involved in the oligogenic inheritance of BBS, in which variants at different BBS loci could modify the severity of the phenotype.(10) Currently, only ten tests available in the Genetic Testing Registry offer *IFT74* sequencing.(11) Of all 373 BBS-related gene panels (including retinitis pigmentosa panels, obesity panels, and kidney panels) there are only nine panels

in the registry that include *IFT74*.(12) Therefore, we recommend *IFT74* sequencing in unsolved cases with the clinical diagnosis of BBS and add this gene to BBS gene panels. This case report also serves as an example of how an exome based approach for diagnostics might be advantageous over a targeted based approach. In this case exome sequencing was performed, but data were analyzed using a virtual gene panel. Using this approach the analysis started with a set of known genes associated with the phenotype of interest and was extended with the analysis of additional genes. In cases where the parents are sequenced as well, the entire exome can be "opened up" to search for novel candidate genes. A virtual gene panel provides a mechanism to include and rapidly add genes of interest and to exclude the analysis of certain genes to minimize the risk of incidental findings.(13) In conclusion, this is the second patient with BBS due to biallelic IFT74 variants, confirming its status as a BBS gene.

Table 1. Comparison of the two IFT74 Bardet–Biedl cases

Feature	**Our case** **11-year-old female**	**Lindstrand case** **36-year-old male**
Primary features of Bardet–Biedl syndrome		
Ocular findings	Rod-cone dystrophy	Retinitis pigmentosa
Postaxial polydactyly	Postaxial polydactyly of the feet	Polydactyly (not further specified)
Truncal obesity	Generalized obesity	Obesity (not further specified)
Learning disabilities/cognitive impairment	Speech delay in childhood, now above average intelligence	Intellectual disability but no developmental delay (sic)
Hypogonadism (in males) or genital abnormalities (in females)	Not present	Hypogonadism
Renal anomalies	Not present	Not present
Other relevant features		
Occipitofrontal circumference	Macrocephaly	Microcephaly
Diabetes mellitus	Not present	Not present
Dental abnormalities	Not present	Not present
Behavioral problems	Not present	Not reported
Craniofacial dysmorphisms	Not present	Not reported
Anosmia	Not present	Not reported
Ataxia	Not present	Not reported

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Acknowledgements
We are very grateful to the proband and her parents for their cooperation and permission regarding this publication.

SUPPLEMENTARY APPENDIX

Table S1. Bardet-Biedl syndrome associated genes tested in our patient as part of an obesity and BBS gene panel analysis

Gene	AR	Associated disorders	Cytogenic location	OMIM
BBS1	AR	Bardet-Biedl syndrome 1	11q13.2	* 209901
BBS2	AR	Bardet-Biedl syndrome 2 Retinitis pigmentosa 74	16q13	* 606151
BBS3 (ARL6)	AR	Bardet-Biedl syndrome 3 ?Retinitis pigmentosa 55 {Bardet-Biedl syndrome 1, modifier of}	3q11.2	* 608845
BBS4	AR	Bardet-Biedl syndrome 4	15q24.1	* 600374
BBS5	AR	Bardet-Biedl syndrome 5	2q31.1	* 603650
BBS6 (MKKS)	AR	Bardet-Biedl syndrome 6 McKusick-Kaufman syndrome	20p12.2	* 604896
BBS7	AR	Bardet-Biedl syndrome 7	4q27	* 607590
BBS8 (TTC8)	AR	Bardet-Biedl syndrome 8 ?Retinitis pigmentosa 51	14q31.3	* 608132
BBS9 (PTHB1)	AR	Bardet-Biedl syndrome 9	7p14.3	* 607968
BBS10	AR	Bardet-Biedl syndrome 10	12q21.2	* 610148
BBS11 (TRIM32)	AR	?Bardet-Biedl syndrome 11 Muscular dystrophy, limb-girdle, autosomal recessive 8	9q33.1	* 602290
BBS12	AR	Bardet-Biedl syndrome 12	4q27	* 610683
BBS13 (MKS1)	AR	Bardet-Biedl syndrome 13 Joubert syndrome 28 Meckel syndrome 1	17q22	* 609883
BBS14 (CEP290)	AR	?Bardet-Biedl syndrome Joubert syndrome 5 Leber congenital amaurosis 10 Meckel syndrome 4 Senior-Loken syndrome 6	12q21.32	* 610142
BBS15 (WDPCP)	AR	?Bardet-Biedl syndrome 15 ?Congenital heart defects, hamartomas of tongue, and polysyndactyly	2p15	* 613580

BBS16 (SDCCAG8)	AR	Bardet-Biedl syndrome 16 Senior-Loken syndrome 7	1q43-q44	* 613524
BBS17 (LZTFL1)	AR	Bardet-Biedl syndrome 17	3p21.31	* 606568
BBS18 (BBIP1)	AR	?Bardet-Biedl syndrome 18	10q25.2	* 613605
BBS19 (IFT27)		?Bardet-Biedl syndrome 19 Bardet-Biedl phenotype: three reports (1-3)	22q12.3	* 615870
BBS20 (IFT172)	AR	Retinitis pigmentosa 71 Short-rib thoracic dysplasia 10 with or without polydactyly Bardet-Biedl phenotype: two reports (4, 5)	2p23.3	* 607386
BBS20 (IFT74)	AR	?Bardet-Biedl syndrome 20 Bardet-Biedl phenotype: 1 previously reported case (6)	9p21.2	* 608040
BBS21 (C8ORF37)	AR	Bardet-Biedl syndrome 21 Cone-rod dystrophy 16 Retinitis pigmentosa 64	8q22.1	* 614477
CEP19	AR	Morbid obesity and spermatogenic failure	3q29	* 615586
TMEM67	AR	{Bardet-Biedl syndrome 14, modifier of} ?RHYNS syndrome COACH syndrome Joubert syndrome 6 Meckel syndrome 3 Nephronophthisis 11	8q22.1	* 609884
CCDC28B	DR/AR	{Bardet-Biedl syndrome 1, modifier of}	1p35.2	* 610162

Other obesity associated genes in the NGS obesity gene panel performed in our case

ADCY3, AFF4, ALMS1, BDNF, CPE, CRHR2, CUL4B, CREBBP, DNMT3A, DYRK1B, EHMT1, EP300, FLOT1, G6PC, GHR, GNAS, HDAC8, IGSF1, INPP5E, IRS1, IRS2, IRS4, KIDINS220, KSR2, LEP, LEPR, MAGEL2, MC3R, MC4R, MCHR1, MEGF8, MKRN3, MRAP2, MYT1L, NDN, NTRK2, PAX6, PCK1, PCSK1, PHF6, PHIP, POMC, PREPL, PRKAR1A, PTEN, RAB23, RAI1, SETD2, SH2B1, SIM1, SNRPD2, SNRPN, SPG11, TBX3, THRB, TUB, UBE3A, and *VPS13B*

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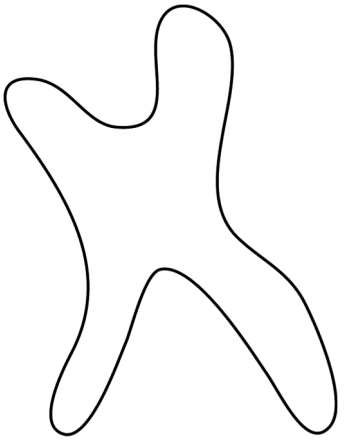
Chapter 7



Who ever heard of 16p11.2 deletion syndrome? Parents' perspectives on a susceptibility copy number variation syndrome

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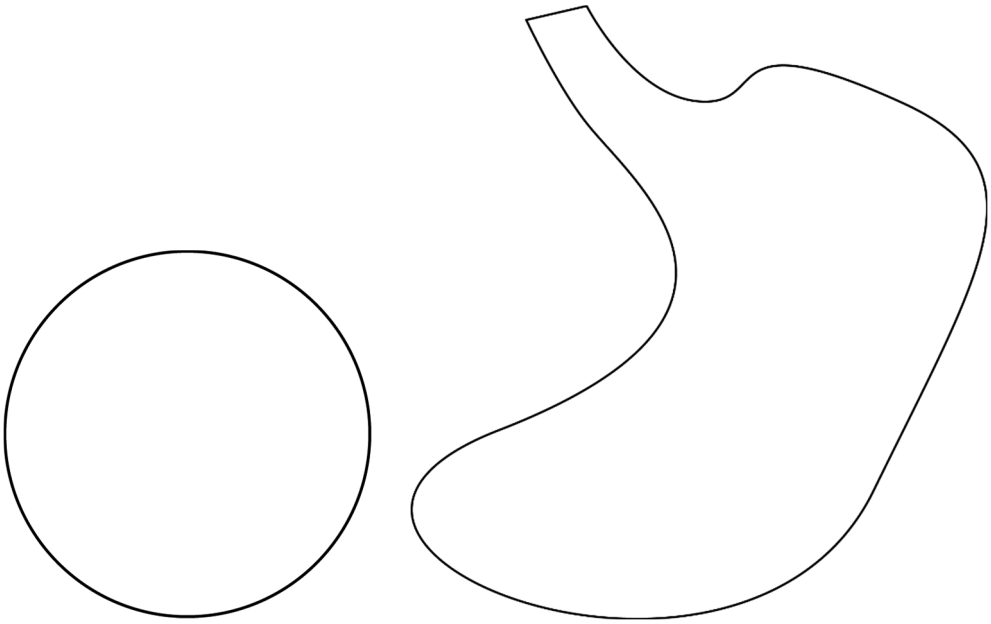

ABSTRACT

Chromosomal microarray analysis is an important diagnostic tool to identify copy number variations (CNV). Some of the CNVs affect susceptibility regions, which means that deletions or duplications in these regions have partial penetrance and often give an increased risk for a spectrum of neurocognitive disorders. Not much is known about the impact of rare CNV susceptibility syndromes on the life of patients or their parents. In this study, we focus on one specific susceptibility CNV disorder, 16p11.2 deletion syndrome. This rare condition is characterised by an increased risk of mild intellectual disability, autism spectrum disorder, epilepsy, and obesity. We aimed to explore the impact of such a disorder on the family members involved in the daily care of children with this syndrome. Three focus group discussions were held with 23 Dutch (grand) parents. Thematic analysis was performed by two independent researchers. The following five themes emerged: (1) the end of a diagnostic odyssey and response to the diagnosis, (2) after the diagnosis—life with a child with 16p11.2 deletion syndrome, (3) access to medical care and support services, (4) nobody knows what 16p11.2 deletion syndrome is, and (5) future perspective—ideal care. The participants experienced a lack of knowledge among involved professionals. Together with the large variability of the syndrome, this led to fragmented care and unfulfilled needs regarding healthcare, social, and/ or educational assistance. Care for children with a CNV susceptibility syndrome could be improved by a multidisciplinary approach or central healthcare professional, providing education and information for all involved professionals.

Introduction

Over the last decades the possibilities for genetic diagnostics have increased enormously, which enables healthcare professionals to provide a diagnosis for patients with previously unexplained symptoms. A consequence of a larger availability of genetic tests is that more patients and doctors are confronted with findings of uncertain significance or diagnoses with a large phenotypic variability. A frequently used genetic test that can lead to complex or uncertain findings is chromosomal microarray analysis, which is a first-tier clinical diagnostic test for developmental delay or congenital anomalies.(1) Assessing the pathogenic effects of the copy number variation (CNV), interpreting the results, and communicating them to the patients and parents can be challenging for healthcare providers.(2) This is especially difficult in the case of CNVs that influence the susceptibility of an individual to specific symptoms or diseases. For many of these so-called "susceptibility CNVs", the main symptoms are developmental delay and psychiatric problems. Some people with a susceptibility CNV do not experience any problems at all. Since many of these rare syndromes have only been recently discovered, most of them are still quite unknown. The most studied CNV is 22q11.2 deletion, which causes the most prevalent CNV syndrome.(3) For 22q11.2 deletion syndrome, a systematic review of studies on the psychosocial impact has shown that parents perceive a lack of knowledge and awareness regarding the syndrome amongst healthcare providers and that they need multidisciplinary care.(4) The increased risk of psychiatric diseases later in childhood appears to be especially challenging to discuss with parents of patients with 22q11.2 deletion syndrome.(5) This is also relevant for other CNVs, since many are associated with an increased risk of psychiatric problems.(6) The second most common microdeletion syndrome 16p11.2 deletion syndrome is much less known and less studied. Little is known about the impact of this syndrome on the life of the patients or their parents, and about their needs and preferences in this regard. Therefore, this qualitative study focused on 16p11.2 deletion syndrome.

16p11.2 deletion syndrome

The name "16p11.2 deletion syndrome" is used for a variety of microdeletions at the 16p11.2 region. Most of the times, the "typical 16p11.2" deletion of ~550–600 kb microdeletion (29.6–30.2 Mb, reference genome GRCh37/hg19) is meant when discussing 16p11.2 deletion syndrome (Online Mendelian Inheritance in Man #611913). It has been shown that 16p11.2 deletion was identified in 1 in 235 in a cohort of over 15,000 cases who underwent chromosomal microarray testing.(3) The deletion is most common in subgroups of patients with autism spectrum disorder; it can be found in 1 in 100 children diagnosed with autism.(7-9) The clinical phenotype is variable, with developmental delay and autism as the most frequently observed characteristics. The majority of the patients will experience speech and language deficits.(10) Around 20% of the patients have epilepsy. Other related medical problems are obesity and vertebral anomalies. Macrocephaly is present in many patients.(11) Although it is an autosomal dominant inherited disorder, most 16p11.2

deletions occur de novo. In the case of an inherited deletion, the clinical phenotype can vary between the affected family members.(9) There are inherited cases where the parent does not show any clinical signs of the 16p11.2 deletion syndrome.(7) In this study, we explored families' perceptions of the impact of a relatively new CNV syndrome, 16p11.2 deletion syndrome. Moreover, we aimed to explore their experiences with healthcare provision and the availability of information. The results of this study will help us to identify the needs of families, which can guide us to pinpoint areas for improvement regarding healthcare and information provision for patients with susceptibility CNVs.

Subjects and methods

Study design

We performed three focus groups with parents and other family members involved in daily care of patients with 16p11.2 deletion syndrome to gain insight into their experiences and perspectives. With the design of focus groups, we expected that this would stimulate a lively discussion among participants and so that we could further explore their different perspectives.(12) The Medical Ethical Committee of the Academic Medical Centre assessed the study protocol and confirmed that the study was exempt from ethics review according to the Medical Research Involving Human Subjects Act (WMO W18-124).

Subjects

Patients, parents, and other caregivers from all over The Netherlands were invited via the Dutch Facebook group for 16p11.2 deletion syndrome and the Dutch society for patients with rare genetic disability disorders (ZeldSamen) to register for the 16p11.2 deletion syndrome information event in 2018. This event has been organised by clinical geneticist (MMvH) and clinical researcher (LK) with the aim to provide the best care for affected individuals by gaining insights in their questions and needs. Based on registration, parents and other family members involved in the daily care of individuals with 16p11.2 deletion syndrome were invited by e-mail to also participate in the focus group study. The focus group interviews were scheduled on an appropriate date and time for participants. Written informed consent was received from all participants prior to the focus group sessions. All 22 families with a child with 16p11.2 deletion syndrome who registered for the event were invited to participate in this study, of which 16 families agreed to participate. Of these 16 families, 23 family members were included in the study (Table 1). When multiple family members of one child applied to participate in the study (*N* = 6 family members), they were placed in different focus groups. This led to a maximum of three participating family members per proband to prevent overrepresentation of certain families and their specific experiences. Participants had a median age of 46 years (interquartile range 45–59 years); 56.5% of the participants were female. The participants were mostly biological parents (19 biological parents, one foster parent, three grandparents). The median age of the children was 9 years (interquartile range 7.3–12.8). All children had the "typical" 550–600 kb

16p11.2 deletion, as well as four of the participating parents. All children of the participants had developmental delay, intellectual disability, or learning problems to various degrees. All except one child attended special education. Seven children had received a diagnosis for a psychiatric disorder including autism spectrum disorder, attention deficit hyperactivity disorder, and depression. Two children were diagnosed with epilepsy. Table 1 shows further sociodemographic and clinical characteristics of the participants included in the focus groups.

Table 1. Sociodemographic and clinical characteristics

Participants (parents/caregivers) *N* = 23	***N* (%)**
Gender	
Female	13 (56.5)
Male	10 (43.5)
Relation to patient	
Biological parent	19 (82.6)
Foster parent	1 (4.3)
Grandparent	3 (13.0)
Age	
20–30	0 (0)
30–40	2 (8.7)
40–50	13 (56.5)
50–60	4 (17.4)
60+	4 (17.4)
Education level[a]	
Low	6 (26.1)
Moderate	8 (34.8)
High	9 (39.1)
Genetic status of the participant	
16p11.2 deletion	4 (17.4)
No 16p11.2 deletion	15 (65.2)
Not tested	4 (17.4)
Participants' children *N* = 16	***N* (%)**
Gender	
Female	8 (50)
Male	8 (50)
Age	
0–4 years	1 (6.3)
4–8 years	3 (18.8)
8–12 years	9 (56.3)
>12 years	3 (18.8)
Genetic status of the child	
Typical 550–600 kb 16p11.2 deletion	16 (100)
IQ[b]	
<70	1 (6.3)
70–79	2 (12.5)
80–89	5 (31.3)
90–109	2 (12.5)
Don't know	4 (25)
Never tested	2 (12.5)
Psychiatric diagnosis[c]	
No psychiatric diagnosis	12 (75)

Autism spectrum disorder	3 (18.8)
ADHD	3 (18.8)
Depression	1 (6.3)
Diagnosed with epilepsy	
Yes	2 (12.5)
No	14 (87.5)

ADHD attention deficit hyperactivity disorder, *IQ* intelligence quotient. [a]Low: elementary school, lower level secondary school, lower vocational training; Medium: higher level of secondary school, intermediate vocational training; High: higher vocational training, university. [b]IQ groups according the Wechsler Intelligence Scale for Children in which 90–109 is an average IQ. [c]Does not add up to 100% because of multiple psychiatric diagnoses per patient.

Data collection

Researchers with expertise in clinical genetics aspects of 16p.11.2 syndrome (LK) and medical psychology (LMvdH) developed a semi-structured topic guide based on the literature and clinical expertise (see Supplemental Material). Topics included the impact of living with a child diagnosed with 16p11.2 deletion syndrome (both on daily life and on psychological and familial functioning) and the experience with and perspectives on healthcare and information provision about 16p11.2 deletion syndrome. Furthermore, participants were asked to complete a short questionnaire on sociodemographic and clinical characteristics directly after attending the focus group. Two out of three focus group sessions were guided by LMvdH as moderator, and one focus group by LK. Each focus group was monitored by an observant. The focus groups were conducted at the Amsterdam UMC, location AMC, Amsterdam, The Netherlands. The sessions lasted 1–1.5 h.

Data analysis

The focus group sessions were audio-recorded and transcribed verbatim. Qualitative data analysis was performed using MAXQDA software version 12.2.1.(13) Thematic analysis was based on the principles of Braun and Clarke.(14) The transcripts were coded by two independent researchers (LK and LMvdH). Any discrepancies between the two researchers were discussed and solved in consensus. The transcripts were read repeatedly to check for consistency between coding analysis and the data. Based on coding analysis, a structure of main and subthemes was created. Data saturation was reached for main themes and most subthemes.(14) Descriptive statistics were used to report participants' characteristics using SPSS version 25.(15)

Results

Thematic analysis revealed the following five main themes: (1) the end of a diagnostic odyssey and response to the diagnosis, (2) after the diagnosis—life with a child with 16p11.2 deletion syndrome, (3) access to medical care and support services, (4) nobody knows what 16p11.2 deletion syndrome is, and (5) future perspective—ideal care. Table 2 shows exemplar participants' quotes per theme to illustrate the results.

Theme 1: The end of a diagnostic odyssey and response to the diagnosis

Most participants reported that many different doctors had been consulted before the diagnosis 16p11.2 deletion syndrome was eventually established. They expressed that they were relieved that they finally knew what was going on with their child when the diagnosis was established (Table 2, Quote 1.1). One participant said that it gave her some peace to having confirmed that her child was "a special child" because this made it easier to accept the child's behaviour and problems (Table 2, Quote 1.2). The child's reaction on the diagnostic process or on the genetic diagnosis was only discussed by a few participants: one of them mentioned that her child asked whether it would be possible to repair the genetic syndrome because the child did not want to have this syndrome and to be different from other children (Table 2, Quote 1.3). One participant mentioned that her 8-year-old child believed that everyone had some kind of a syndrome (Table 2, Quote 1.4), thus having 16p11.2 deletion syndrome is not that special or interesting because every single person has "something" that makes him or her unique.

Theme 2: After the diagnosis—life with a child with 16p11.2 deletion syndrome

Burden on the family

Medical problems, such as obesity, constipation, frequent ear–nose–throat infections, and sleeping problems, were reported by several participants. Many participants experienced difficulties in looking after their children and provide the best upbringing (Table 2, Quote 2.1). Most participants felt that taking care of their child takes a lot of effort; only two participants disagreed and said that it was comparable to the upbringing of their other unaffected children. One participant said that the daily care is extremely intensive and that he had to watch him constantly. Many participants experienced a lack of satiety in their children, even after eating. Some participants mentioned that they found it hard to set boundaries related to food, because it made their child unhappy. Some participants told that they did not buy certain types of unhealthy food because the child would secretly eat food. A few participants mentioned a discrepancy between the child's behaviour at home and at school (Table 2, Quote 2.2). At school or with family and friends, they behave properly, but at home some children had tantrums or became verbally aggressive towards parents and siblings. These differences in behaviour at home versus at school were hard to understand for the parents. The diagnosis has led to changes in life choices for some participants; some decided not to have another child, another participant decided not to move abroad (Table 2, Quotes 2.3 and 2.4).

Worries about the child's social life and place in society

Problems in communication were frequently reported by the participants. Most of the participants expressed that it was difficult for them when they were not able to understand their own child (Table 2, Quote 2.5). It was sometimes reported to be difficult in

communication with friends or classmates as well (Table 2, Quote 2.6). One participant mentioned that other children sometimes think that his son speaks a foreign language, because they cannot understand him. For several children the communication problems resulted in having no or limited contact with their peers. Some participants also mentioned that their children were frequently unable to participate in regular social or sports activities often because of their fatigue and communication problems. Their increased appetite also led to problems at school for some children. There was a teacher who thought that the child did not receive enough food (even though the child had obesity); whereas another teacher was worried about the child's obesity and suggested not to give birthday treats to the affected child any longer (Table 2, Quotes 2.7 and 2.8). Participants reported that school days were generally too long for children, due to large travel distances between home and special education services. Travelling to school requested additional support from some participants. Many children were generally not able to travel to school alone, in contrast to what teachers at school sometimes expected. For other children, no arrangements for travelling to school were available. Multiple participants worried about the ability of their child to do things independently and to live on their own in the future.

Administrative and financial burden

Many participants experienced the financial and administrative issues related to the support of their child as a burden. The awareness of financial support and the possibilities for support available per child was different between the municipalities where the participants lived in. In The Netherlands, most of these children are eligible for the "individual budget" (budget provided by the government that parents can apply for to arrange and purchase assistance for their child). To receive this budget, the participants often had to prove that their child still needed this additional support. Participants found this frustrating, since once a diagnosis is established, this does not change over time (Table 2, Quote 2.9). Moreover, they felt that the officials judging the application did not understand what the 16p11.2 deletion syndrome exactly means (Table 2, Quote 2.10). Some participants mentioned that they did not receive any financial support at all for their child's healthcare and support. This lack of financial support surprised another participant, because her municipality even paid for professional help to teach her child how to ride a bicycle.

Theme 3: Access to medical care and support services

The participants mentioned that many different healthcare professionals are involved in the treatment or care of their children. For example, a majority of the patients visits or visited a speech therapist. Many patients also visited a dietician. The majority of participants experienced limited access to support or care. There were few participants who reported that their child received no medical care or support at all (Table 2, Quote 3.1). There was only one participant who told that there was no need for any treatment or support at that

time (Table 2, Quote 3.2). Others put much effort and time into requesting care but did not always manage to receive it. These participants were unsure why they did not receive the requested care, but argued that it could be related to the unfamiliarity and variability of the syndrome.

Theme 4: Nobody knows what 16p11.2 deletion syndrome is

Having a well-known syndrome would be easier

One participant told it was unclear for them and their children what 16p11.2 deletion syndrome meant and that the name of the syndrome was too difficult for them (Table 2, Quote 4.1). Another participant mentioned that their child did not look different from unaffected children. As a result, other people, including professionals, generally do not understand that these children need additional care and support. Since 16p11.2 deletion syndrome is a rare syndrome that has been recently discovered, many participants felt that the syndrome was relatively unknown, and not all symptoms and characteristics of this condition were clear to healthcare professionals. Participants therefore believed they had to sort out many things themselves, and that it was unclear where to get support or that support was not adequately provided. Some participants therefore believed that it would have made life easier if their child would have been diagnosed with Down syndrome, which was considered a better-known syndrome, instead of 16p11.2 deletion syndrome (Table 2, Quote 4.2). One participant had experienced a lack of knowledge about 16p11.2 deletion syndrome among all involved healthcare providers. More participants observed this lack of knowledge amongst teachers and education professionals involved in the care of their children. Some participants considered teachers incapable of educating this "type" of children because they did not understand what 16p11.2 deletion syndrome involved and how symptoms should be handled and had not read the information about the syndrome that the participant had given them (Table 2, Quote 4.3). However, others were very satisfied with the school and the teachers. Many participants said that their information needs were unmet. Some participants knew about online disorder information, such as the Unique guides or Facebook pages, to find more information about the syndrome. Others were unaware of the available information or desired more information than available. They also expressed the need to share experiences with other parents or caregivers. Information specifically for affected children themselves was perceived as still lacking.

16p11.2 deletion syndrome is variable which leads to uncertainty

Many participants reported that the variability in symptoms and related consequences of 16p11.2 deletion syndrome caused uncertainty. The participants discussed that they had to monitor themselves whether or not symptoms were developing in their child, for example whether their child was gaining weight (Table 2, Quote 4.4). Another participant mentioned that the variability of behavioural and psychiatric problems as part of 16p11.2 deletion syndrome was difficult to interpret: the participant was uncertain whether his child's

behaviour was related to the syndrome or just his personality (Table 2, Quote 4.5). The variability and uncertainty regarding the intellectual capacity of children with 16p11.2 deletion syndrome was discussed as well. For some children regular education was suitable, while for others special education was needed. Participants with younger children expressed the need for guidance regarding the most suitable school (type) for their child.

Theme 5: Future perspective—ideal care

After hearing the genetic diagnosis it still remained difficult for some of the participants to receive adequate healthcare and information. For others, the diagnosis had opened doors to appropriate healthcare. Participants mentioned that they would feel supported if there would be personalised or standardised treatment available to relieve the symptoms associated with 16p11.2 deletion syndrome. Some participants noted that it would be better if there was a main healthcare provider to coordinate care or a central place where they could ask questions regarding the syndrome, for example a specialised outpatient clinic (Table 2, Quote 5.1). Two participants mentioned that they would prefer to hear more from doctors and researchers about the experiences with medication in patients with 16p11.2 deletion syndrome. The participants also wanted to ask non-medical questions to these 16p11.2 deletion syndrome experts, for example which school type is most suitable for their child. Several participants mentioned that professional assistance in applying for financial support would be helpful as well (Table 2, Quote 5.2).

Table 2. Exemplar quotes per theme

Quote number	**Focus group (FG), participant (P)**	**Quote**
Theme 1: The end of a diagnostic odyssey and response to the diagnosis		
1.1	FG2, P4, F1	"We were very relieved then [with the diagnosis]. We were visiting doctors for seven years until we knew what it was. So we were sort of relieved, you know, that we were not crazy. That child does have something".
1.2	FG1, P3, F2	"I have said, for my own sake and for her interest, this is how she is. Yes a special child, and that gives a parent peace".
1.3	FG2, P3, F3	"He wants it to be fixed, that the gene will be repaired (…). They should find a medicine for it".
1.4	FG2, P1, F4	"[Child] thinks everyone has a syndrome. (…) She said 'mommy I'm glad that I don't have my brother's syndrome' - Her brother has a terrible morning mood at the moment - 'I'm glad I have 16p syndrome".
Theme 2: After the diagnosis—life with a child with 16p11.2 deletion syndrome		
2.1	FG2, P2, F5	"Yes, it makes it a very intangible disease and if you look at the impact on our social life… When we go somewhere I always check [the surroundings], and my wife does so as well. We look around 'this could fall down', 'he could fall on his face over there', he will take the television of the wall so to speak".
2.2	FG2, P1, F4	"[My child] looks way too pretty. She looks too good, behaves well outside the house, but at home…".

2.3	FG2, P3/P4, F3/F1	"P3- I say this very honestly, I am happy that we only had one child in our case. Because, I'm very glad with this boy, because it's a very sweet boy. But I, I couldn't have handled a second one, whether or not he would have it [16p11.2 deletion syndrome] or not (…)" – P4: Yes, I recognise that, we have two [children] and the youngest has this. And my partner really wanted a third [child], but I [said] no. Indeed what you said, I can't handle that".
2.4	FG3, P9, F6	"We once said, we would like to live abroad for a couple of years (…) but the healthcare and schools and support… It is so important to have that (…). So maybe that's the reason not to go".
2.5	FG3, P4, F7	"It is quite frustrating sometimes when you do not understand your own child".
2.6	FG1, P8, F8	"He [child] also can't communicate and he doesn't have any friends. For himself, he does have friends, but the friends don't regard him as a friend".
2.7	FG1, P7, F9	"When there is a birthday, this may sound weird, I can always find her in the kitchen, begging for food like a dog".
2.8	FG2, P6/P2, F10/F5	P6 "And in school you get complaints. You give so little food to your child? That kid needs more food. Yes, but she cannot [get more food]!". P2: "Yes but in the old school of [my child] it was the other way around. The teacher called to ask whether we should skip [child] with birthday treats. I found that very sad".
2.9	FG1, P1/P6, F4/F11	P6: "That is the problem, we are busy with the individual budget again, but you have to go there every year, that is horrible and I hope you can change that". P1:" Yes because it doesn't change. Their chromosomes will never change (…), but every time we have to explain this again".
2.10	FG2, P1/ P2, F4/F5	P1: "Yes the individual budget. The enormous fight (…) I become very frustrated that she constantly has to be tested by people who don't understand what she has". – P2: "Yes you are at the mercy of the whims of bureaucracy".
Theme 3: Access to medical care and support services		
3.1	FG1, P7, F9	"I receive no support, no help. They told me my daughter has it [16p11.2 deletion syndrome] in 2015 (…), I asked [the clinical geneticist]: Who I can talk to about the problems we keep running into? Who can help me? - I still know nothing about this".
3.2	FG2, P9, F12	"We don't need any help, the only thing we have is that school once asked for extra support, but we don't have any other financial help or anything, and that works great".
Theme 4: Nobody knows what 16p11.2 deletion syndrome is		
4.1	FG2, P5, F13	"Come on, that [16p11.2 deletion syndrome] is not a nice name for the syndrome. Couldn't they invent something that children can pronounce as well?"
4.2	FG2, P1/P4/P5, F4/F1/F13	P4: "A girl on the bus has Down [syndrome], she gets everything [support]. But [child] looks nice, is a pretty girl, that's a pitfall". – P1: "I also often wished she had Down [syndrome]. Purely because it would be easier". – P5: "And there are guidelines for it, but for 16p there is nothing".
4.3	FG1, P7, F9	P7: "16p, I think they [the teachers] never read it [the information]".- Moderator: Did you give [the school] the information? – P7: Yes, yes, I told which syndrome she has. Read it, acquaint yourself with it!".
4.4	FG3, P9, F6	"We really need to find a balance, I don't expect that he will get it [obesity]. But it could start from the age of ten – I first heard it would start at age seven, now at age ten – so he could develop it [obesity], but I don't expect it".

4.5	FG2, P5, F13	"It is difficult to say what is part of [his] personality and what has to do with the syndrome".
Theme 5: Future perspective—ideal care		
5.1	FG1, P7, F9	"What I miss most is someone or an outpatient clinic or something like that, where I can tell my story to someone who knows what the syndrome is".
5.2	FG3, P5, F14	"I think it's a shame that you have to figure it all out by yourself, that you can apply for the individual budget, for a public transport companion pass, you all need to hear that from someone else who tells you what's possible".

Discussion

This focus group study gave insight in the perspectives and experiences of (grand)parents on having a child with 16p11.2 deletion syndrome and the impact of this particular CNV syndrome on their child's and family's lives. Our participants reported that their children experienced many medical and psychosocial problems impacting daily life, and described the challenges for parents raising their children. The stories of the participants uncovered a variety of important themes that, to our knowledge, have not been discussed in the literature before in the context of 16p11.2 deletions or similar susceptibility CNVs.

Our participants felt relieved once the diagnosis for their child was established. This "end of a diagnostic odyssey" is a well-known term in the genetic literature.(16) Receiving a genetic diagnosis can be very helpful for parents, since this will give insights in the symptoms or intellectual development they can expect for their child, and comorbidities associated with the disease can be monitored. The benefits of knowing the genetic diagnosis were mentioned by our participants, but appeared to be less evident for them in the long term. The unpredictability whether their child will have learning problems or develop symptoms such as obesity is difficult to handle for parents. In fact, experiencing uncertainty following the diagnosis is a common problem with CNVs.(17, 18) This uncertainty has multiple causes: there is often incomplete penetrance of the phenotype for many CNVs, there is often a large variability of symptoms, and there is (still) a lack of information about the prognosis of the disorder.(3, 19) Moreover, uncertainties about the future are frequently reported by caregivers of patients with chronic diseases in general.(19) Another extensively discussed topic during the focus group meetings was the financial and administrative burden that the parents experience. These findings are in line with the literature on the experiences that parents of children with rare diseases in general have, feeling burdened by their role as care coordinator.(20)

Our study also aimed to explore the experiences of parents with the healthcare and information provision. Many of the participants in the current study did not feel sufficiently supported by healthcare providers to learn about the disorder and its associated symptoms. Parents participating in the current study experienced that they were the 16p11.2 deletion syndrome experts instead of the doctors, especially since many believed that the healthcare providers were unable to give them sufficient information about the syndrome. This is in

line with the literature about parents of children with rare diseases, who feel that they are the "expert caregivers" and know more about the disorder than the involved medical specialist.(20) It is possible that the parents did receive adequate counselling about the disorder, but that the parents were not able to obtain the information at that moment. Timing in the delivery of information is therefore important. Moreover, the parents' questions and information needs can change during the development of the child. Repeat healthcare visits to gain information about the disorder during childhood and adolescence can be helpful to address these questions. Although certain information is available for parents, not all participants in our focus group were aware of this. For example, charity organisation Unique offers disorder guides for many rare chromosome disorders to inform both parents and healthcare professionals (www.rarechromo.org). Some participants mentioned that the difficult name of 16p11.2 deletion syndrome is not beneficial for the general knowledge and awareness of this genetic condition. This will apply to many other deletion or duplication syndromes as well. Parents' wish for a simpler name is important to note because of the trend to name genetic disorders on a description of the disorder and the underlying genetic cause.(21)

The medical problems associated with 16p11.2 deletion syndrome are diverse and participants mentioned that many different healthcare providers were involved in the treatment of their children. Moreover, they experienced that current healthcare and support is very fragmented. This made them feel that access to care and adequate treatment was restricted. A large literature review of parents that have a child with a chronic disease showed that these parents also experienced difficulties in obtaining information and were unsatisfied with the information provided by healthcare professionals.(22) Indeed, many medical professionals acknowledge that organizing medical care for patients with very rare disorders is challenging and needs to be arranged in centres of expertise.(23) Having one main healthcare provider or coordinator of a multidisciplinary team could improve the medical support for children with CNV syndromes and their parents, in which the central healthcare professional provides information about the syndrome to all involved professionals. We think that the input of parents, expressing their concerns and wishes, is necessary to shape this multidisciplinary healthcare team. This thought is supported by previous findings that parents of children with rare diseases find it important that healthcare providers stimulate them in their active participation regarding their child's healthcare.(19) Healthcare providers should also stimulate peer support for these parents. Because of the rarity of these syndromes, it can be difficult for parents to find other parents who are facing similar problems. We would recommend doctors to stimulate parents in visiting patient information events or to refer to disorder support groups on social media.

This study gives new insights in the perspectives of parents regarding 16p11.2 deletion syndrome and microdeletion/duplication susceptibility syndromes in general. A limitation of this study is that we could only perform three focus group interviews because of the relative rarity of the syndrome. However, data saturation was reached for most themes and subthemes. Moreover, the fact that our participants visited a patient information evening and wanted to participate in this study might have led to a bias because they either experienced more problems regarding the syndrome or because they were more interested in patient advocacy and parent support. Our described group of children seems to be representative for patients with 16p11.2 deletion syndrome in terms of IQ and other characteristics of 16p11.2 deletion syndrome, although this is difficult to assess with small numbers. However, in our patient group most children did not have a psychiatric diagnosis, whereas the literature reports that a majority of individuals with a 16p11.2 deletion have one or more psychiatric diagnoses.

This qualitative study is the first to explore the experiences and perspectives of parents with a child with 16p11.2 deletion syndrome. The findings of this study offer interesting opportunities for future research. One of our findings that is quite specific for 16p11.2 deletion syndrome is that some parents reported they needed more support to cope with their child's increased appetite and food behaviour problems. It would be useful to explore this topic further in the future, possibly by learning from other genetic disorders with increased appetite such as Prader–Willi syndrome. Furthermore, research focusing on improving the coordination of care for these families would be recommended. Although 16p11.2 deletion syndrome is relatively rare and the numbers to include are therefore expected to be relatively small, a survey study to assess parents' experiences and psychological impact of having a child with 16p syndrome, incorporating the findings of the current study, would be interesting to further investigate and quantify our findings on group level. In a future study, we also aim to explore the perceptions of patients themselves as well, although this might be difficult to arrange because of the large variability in age and intellectual abilities.

In conclusion, parents reported a lack of understanding and information regarding the syndrome and its associated variability amongst both healthcare providers and other involved professionals, which—for many parents—resulted in fragmented care and support. The 16p11.2 deletion syndrome is quite recently discovered and still unknown to many doctors, even though it is the second most common microdeletion syndrome. Because of new and more commonly used diagnostic genetic techniques, novel syndromes are discovered on a regular basis. It is therefore of vital importance that clinical geneticists and genetic counsellors assess the information needs regarding these syndromes to adequately inform both patients and other involved professionals. The presented findings might be applicable for other susceptibility CNV syndromes as well. This can be used to

improve our healthcare for patients with these syndromes and their families. Based on the findings we make the following recommendations for clinical practice: **1.** Many participants experienced a lack of information about 16p11.2 deletion syndrome. Repeat visits can be useful during childhood and adolescence to address new questions and needs. This also enables the healthcare provider to divide the extensive information over multiple visits and to repeat provided information, taking the age and developmental phase of the patient into account; **2.** doctors can also help to improve information provision by creating awareness of the available guides for rare genetic disorders and other ways to receive up-to-date information such as organising patient information evenings or participating in online support groups for parents; and **3.** because of the large variability of symptoms in 16p11.2 deletion syndrome, patients are often treated by many different healthcare providers. A personalised and centralized multidisciplinary approach in both medical treatment as well as psychosocial and educational support is therefore needed.

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SUPPLEMENTARY APPENDIX

Topic List Focus Group with Parents of 16p11.2 Deletion Patients

Study Introduction

1. Welcome
2. Introduction of moderator
3. Explanation study goal
4. Explanation process of the focus group interview, there are no wrong or right answers, estimated duration of the focus group interview
5. Explanation role of the moderator
6. Information about audio recording, consent procedure
7. Questions before start of the study?

Introduction round

1. Name
2. Reason to participate

Experiences, impact and coping with 16p11.2 deletion

1. Symptoms/complaints: physical complaints (constipation, infections etc.), fatigue, development, sleeping problems, cognitive problems, language and speech
2. Impact of 16p11.2 deletion syndrome on parent's life (feelings, life choices, coping, support, impact on daily life)

Behaviour

1. Character, social behaviour, interactions with peers or siblings, behavioural problems
2. Behavioural/psychiatric diagnosis (impact, does this help understanding)
3. Consequences of behaviour (social impact, interaction with siblings, place in family, peers, bullying, activities, lifestyle)
4. Factors influencing behaviour (setting, life phase, medical problems such as pain or fatigue)
5. Satiety/hyperphagia (dealing with hyperphagia, support)

Experience with and need for support from healthcare providers

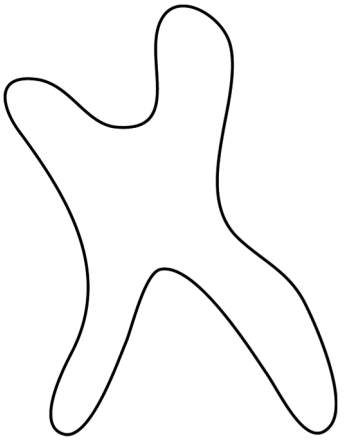
1. Which support do you currently receive from healthcare providers? Societal effects of 16p11.2 deletion syndrome (financial support, education, medical aids)
2. What are your experiences with healthcare providers and/or medication?
3. What do you think of the information you received about 16p11.2 deletion syndrome? (important information, missing information, best way to deliver information, views on peer or parental support groups)

4. Which support/treatment/therapy do you find helpful/useful/good for you and your child? Are there things that did not work for you? What would you want to change?

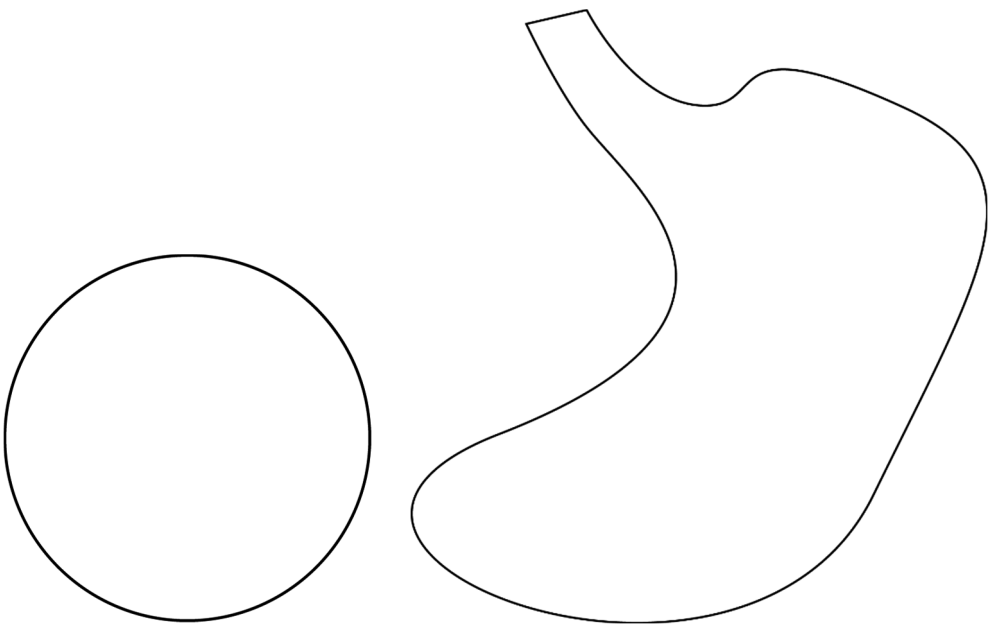
Conclusion

1. Comments or questions?
2. Thanks for contribution, instruction short questionnaire on sociodemographic and clinical characteristics

Chapter 8



Summary



SUMMARY OF THE THESIS

Genetic Obesity: Disorders and Diagnostics

Part I - DNA diagnostics for rare genetic obesity disorders

Over the past 25 years, enormous progress has been made in the diagnostics of rare genetic obesity disorders. At first, multiple consecutive Sanger sequencing tests were needed to establish a diagnosis, which was a time-consuming and costly process. In 2012, our research group designed one of the first next-generation sequencing gene panels for genetic obesity. **Chapter 2** describes the results of obesity gene panel testing in 1230 children, adolescents, and adults with obesity. We were able to establish the diagnosis of a rare genetic obesity disorder in around 4% of the patients, which was higher than in previous studies of non-consanguineous patients with obesity. Furthermore, we show that genetic obesity gene panels need to be comprehensive, since we established diagnoses based on mutations in 11 different genes. The highest diagnostic yield can be achieved in subgroups of children with early-onset obesity. **Chapter 3** displays the yield of the same gene panel test performed in 1014 patients who underwent bariatric surgery. In this subgroup, the diagnostic yield was lower and less heterogeneous, as pathogenic mutations were only identified in five different genes.

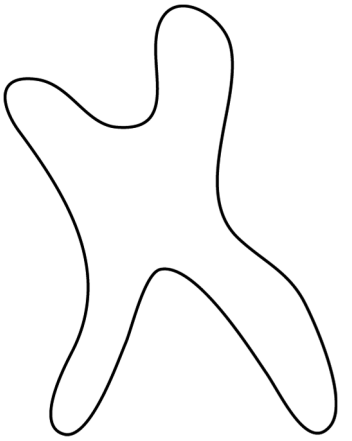
Part II - Extensive phenotyping to distinguish rare genetic obesity disorders from common obesity

Because of the high prevalence of obesity, it is impossible to perform genetic diagnostics in all patients with (severe) obesity. An improved insight in the clinical phenotype of patients with a genetic obesity disorder is therefore needed to determine which patients should undergo genetic testing. In **chapter 4**, we display our systematic diagnostic approach to identify the underlying medical causes of pediatric obesity. This approach comprises not only genetic causes of obesity, but also endocrine, cerebral, and medication-induced underlying causes of obesity. We show that this systematic phenotype work-up helped us increase the diagnostic yield; we were able to find singular underlying causes in 19% of our patients with severe childhood obesity. Moreover, we could identify some clinical indicators for genetic obesity disorders. In **chapter 5**, we present an overview of the phenotype of a specific genetic obesity disorder, leptin receptor deficiency. Because of its great variability, we hypothesized that leptin receptor deficiency is an underdiagnosed disorder. Based on carrier frequency data from 77165 European individuals, we estimate that leptin receptor deficiency is much more prevalent than currently known. As multiple clinical trials with novel drug therapy for this disorder are currently performed, diagnosing leptin receptor deficiency is more important than ever.

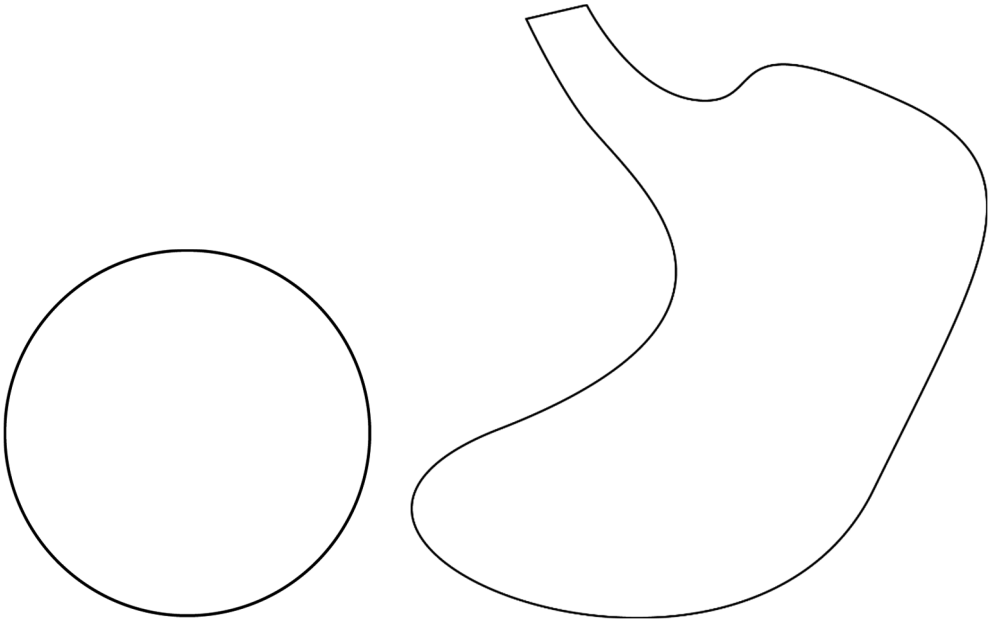
Part III - The Importance of a Diagnosis in Rare Genetic Obesity Disorders

In part 3, we aim to show the importance of the diagnoses described in the previous two parts. In **chapter 6**, we present four case reports on different genetic obesity syndromes. The first article describes the case of a girl with severe early-onset obesity and hyperphagia, in which the genetic diagnosis of leptin receptor deficiency caused a dramatic change in BMI. In the second article of **chapter 6**, a girl with Schaaf-Yang syndrome is described who died at the age of 23 months. We state that strict monitoring and treatment of obesity in patients with Schaaf-Yang syndrome is needed, since obesity and the syndrome-associated pulmonary problems, hypotonia, and apnea can be a fatal combination. In the third article, we report a case of PTEN hamartoma tumor syndrome, which shows the possible serious consequences of genetic analysis in the bariatric clinical practice. In our last case report in **chapter 6**, we present an 11-year-old girl with the clinical diagnosis Bardet–Biedl syndrome. We identified biallelic variants in *IFT74*, a gene that was reported only once before as a Bardet-Biedl gene. Based on this finding, we recommend adding *IFT74* to Bardet-Biedl syndrome gene panels. We conclude part 3 with a qualitative study in **chapter 7**, which explores the experiences and perspectives of parents who have a child with the syndromic obesity disorder called 16p11.2 deletion syndrome.

Chapter 9



General discussion



DISCUSSION

When we try to explain obesity at the most basic level, it appears straightforward: obesity develops when people consume more energy than they expend. Our obesogenic environment, with plenty of energy-rich foods and little physical activity, works as the ideal incubator for developing obesity. However, obesity is much more complex. Twin and adoption studies have shown that that the heritability of obesity is relatively high in our environment, which means that a substantial proportion of variability in the trait obesity between people is caused by genetic factors.(1) Many people with obesity will have a combination of genetic factors that make them more susceptible to developing obesity, but it is currently not possible to determine all these possible factors. This is different for rare genetic obesity disorders, in which one genetic defect is the main cause of the obesity. These disorders and the diagnostic options to identify them are extensively described in the previous chapters. In this section, the results of these studies are discussed. Moreover, we propose future research ideas and implications for current clinical practice.

How next-generation sequencing changed genetic obesity diagnostics

In the 1970s, the first methods for DNA sequencing emerged, of which the technique developed by Frederick Sanger was most successful.(2) For thirty years, Sanger sequencing was the most important diagnostic tool for monogenic diseases. In the 2000s, a new era started with the use of high-throughput sequencing technologies that enable parallel testing of multiple genes. DNA diagnostics became cheaper and less time-consuming than before. Moreover, next-generation sequencing (NGS) accelerated the identification of dozens of novel disease-causing genes.(3) **Chapter 2**, "Genetic obesity: next-generation sequencing results of 1230 patients with obesity", was one of the first articles reporting the results of massively parallel sequencing for genetic obesity disorders. In **chapter 3**, we present the clinical use of this NGS gene panel in the bariatric clinic. The most important findings of these two articles were that the diagnostic yield for rare genetic obesity disorders is the highest in subgroups of children with severe early-onset obesity. The diagnostic yield in the bariatric clinic however, is much lower, even with a selection of patients who had a BMI over 50 kg/m^2 or with a history of early-onset obesity. In the near future, we hope to further improve these selection criteria, especially because of the hopeful prospect of targeted treatment for several genetic obesity disorders.(4)

In **chapter 2** and in **chapter 4**, we also demonstrated that the heterogeneity of obesity requires sequencing of multiple genes to diagnose rare genetic obesity disorders. This finding has important implications for the design and clinical use of obesity gene panels. The current clinical practice guideline for pediatric obesity by the Endocrine Society suggests genetic testing in patients with symptoms suggestive of a genetic obesity disorder.(5) However, they do not recommend which test should be performed when

considering molecular genetic studies. Since the phenotypes of the leptin-melanocortin pathway disorders are quite similar, we advise against single gene analysis for patients suspected of these disorders. If available, once should consider performing an NGS gene panel consisting of all important genetic obesity genes or a whole exome sequencing (WES) based gene panel. WES-based gene panels offer the additional possibility for reanalysis when novel obesity genes are discovered after the initial gene panel test was performed. Moreover, clinicians should always keep in mind that several disorders that are associated with obesity, such as Prader-Willi syndrome or Fragile-X syndrome, cannot be diagnosed through these NGS gene panels. Especially for patients with intellectual disability and a history of neonatal hypotonia, specifics tests for methylation defects can be indicated.

An important downside of extensive multigene-panel testing is the increased chance of identifying carrier statuses of an autosomal recessive condition. Over 11% of the 1230 patients described in **chapter 2** were indeed carrier of a heterozygous (possibly) pathogenic variant in a gene associated with an autosomal recessive inherited condition. The possibility of finding a carrier status should either be counselled before ordering the test, or genetic testing laboratories should choose to refrain from reporting heterozygous VUS in genes associated with recessive disease in non-consanguineous patients. Furthermore, extensive genetic testing also makes it more likely to identify variants of uncertain significance (VUS). When VUS are found, the patient should be referred to a clinical geneticist for further clinical evaluation and to determine whether segregation analysis might help to elucidate the pathogenicity of the variant. In some countries, there is discussion who should cover the costs of further testing of VUS in family members. Because of the clinical implications for the patient, we believe that segregation analysis should be covered by the patient's health insurance.

Phenotyping is complex but important

Obesity is a heterogeneous disease in both phenotype and genotype and its underlying mechanisms range from monogenic to complex or mainly environmental. Therefore, it will remain difficult to identify the molecular causes for individual patients. We believe that phenotyping, although complex, still plays an important role in selecting patients that should undergo a genetic test. Moreover, detailed phenotyping is essential to interpret the results of genetic testing. In **chapter 4**, we showed that careful examination of the patient's medical history, laboratory analysis, family history, and growth curve is needed to identify the possible underlying causes of obesity. At our tertiary obesity center for children, we use this approach to evaluate all patients for the medical causes of obesity as mentioned in the ES guideline: endocrine and genetic disorders, as well as cerebral injury and use of obesogenic drugs.(5) This resulted in a much higher diagnostic yield (19%) than previously reported.(6, 7) Furthermore, we found that onset of obesity before the age of five and hyperphagia were statistically significant indicators of underlying genetic causes, but only

in patients without intellectual disability. Patients with syndromic obesity disorders more often had a history of neonatal feeding problems and a short stature. Growth curve analysis proved to be a very valuable tool to gather objective evidence for a medical cause of obesity. A clear slope discontinuity in the growth curve after an ischemic event or after the start of obesogenic drug treatment helps to identify these underlying causes. Furthermore, detailed assessment of the growth curve to determine the age of onset of obesity can indicate whether genetic testing is needed. We are currently working on a more extensive analysis of the BMI trajectories of children with severe early-onset of obesity. This will hopefully result in an algorithm to identify rare genetic obesity disorders in an earlier stage of the disease.

The systematic assessment of the patient's phenotype, in combination with the family history, is pre-eminently a task for the clinical geneticist. Therefore, we believe that clinical geneticists should be involved in the diagnostic process for rare genetic obesity disorders as part of a multidisciplinary medical team with other specialists such as (pediatric) endocrinologists, dietitians, and physical therapists. Moreover, clinical geneticists should be involved in the development of protocols for the diagnostic approach of genetic obesity. We hope that this will improve the recognition of these rare disorders. This is needed because personalized medicine options will further develop in the nearby future. Moreover, some of the monogenic obesity disorders might be more prevalent than currently known. As we stated in **chapter 5**, it appears that only a small minority of the predicted European cases with leptin receptor deficiency are currently recognized. This phenomenon is also described in for other leptin-melanocortin pathway disorders.(8) **Chapter 5** further gives an overview of the phenotype of all currently known patients with leptin receptor deficiency. We found that the median age of onset of obesity in these patients was 0.3 years and that the typically disease-associated pituitary hormone disturbances were only present in 34% of the patients. These findings support our opinion that every child with an extreme early onset of obesity should undergo genetic testing. One of the main knowledge gaps for leptin receptor deficiency is the clinical course in adulthood. In some cases, improvement of the endocrine phenotype has been reported. Regarding the weight trajectories, only case reports have been published about the treatment results in a small number of patients. Long-term follow-up studies of patients with leptin receptor deficiency are necessary to better inform patients and their families about what they can expect in the future. Our extensive literature study and similar studies for other genetic obesity disorders are needed to make clinical management guidelines. These guidelines should make recommendations for the standard evaluations following initial diagnosis and for surveillance protocols.

Why a diagnosis matters

In the third part of this thesis, we illustrate the importance of diagnosing genetic obesity disorders. We described five different genetic obesity disorders: leptin receptor deficiency,

Schaaf-Yang syndrome, PTEN hamartoma tumor syndrome, Bardet-Biedl syndrome, and 16p11.2 deletion syndrome. The most important message from these articles is that the social stigma towards obesity is burdening and that a genetic diagnosis can change the lives of these patients even when disease-specific treatment options are not available (yet). One of our articles that was most frequently discussed at international meetings was, surprisingly enough, a case report. In the article "Young girl with severe early-onset obesity and hyperphagia", presented in **chapter 6**. The mother of this patient gives voice to parental frustrations and fears regarding the child's severe obesity. Furthermore, we show that the diagnosis and subsequent advices helped them to achieve weight stabilization. For this girl, and many other patients, there is even more hope on the horizon. In 2016, MC4R agonists were shown to have a positive effect on the BMI and hunger scores of two patients with biallelic *POMC* mutations.(9) Successful results were later found for leptin receptor deficiency as well.(10) Larger clinical trials will probably elucidate the potential of these drugs in more genetic obesity disorders in the following years. Besides the development of new drug therapies, there has to be more focus on studying and improving the current supportive care for these patients. As is true for many rare diseases, (inter)national collaboration will be necessary to find the most effective supportive treatment. This would include dietary advices, psychological help, and parental coaching for coping with their child's hyperphagia. More research is currently performed on bariatric surgery options for rare genetic obesity disorders, but it is still unclear which patients will or will not benefit with long-term weight loss.(11) We are awaiting the results of large and long-term follow-up studies for patients with the most common genetic obesity disorder, *MC4R* deficiency, who underwent gastric bypass or sleeve operations. For bariatric surgery, efforts should be made to provide pre-operative obesity genetic test results. Receiving results that negatively affect surgery outcome could prevent that affected patients will undergo risks of a suspected unsuccessful operation.

Future diagnostics in clinical practice

The study of these rare genetic obesity disorders can lead to fundamental insights that might be applicable to common obesity. This can easily be seen when looking at *MC4R*, the gene that is most frequently involved in non-syndromic genetic obesity. This gene also plays a role in polygenic obesity, as several polymorphisms have been identified that are either protective or risk factors for obesity.(12) Multiple genome wide association studies have identified a large number of obesity susceptibility loci that contribute to polygenic or common obesity. To determine the combined effects of these variants in individuals, genome-wide polygenic risk scores for obesity are being developed. The first large-scale studies on polygenic obesity risk show that adults with high polygenic risk score of obesity have a 25 times higher chance of having severe obesity than the individuals with a low risk.(13) Furthermore, a low polygenic obesity risk can sometimes even neutralize the obesogenic effects of pathogenic *MC4R* mutations.(14) Therefore, polygenic risk scores

might partly explain the low penetrance or variable expression of rare genetic obesity disorders. We showed in this thesis that there are individuals with a known pathogenic variant in *MC4R* who are lean, whereas their children with the same pathogenic variant suffer from early-onset obesity. When designing a polygenic risk prediction model, the intended use of the model has to be taken into account. If we want to advance our clinical health practice, the implementation of a polygenic risk score will be the most useful when we combine the scores with data on treatment success. However, there is an important question that we do need to ask ourselves before implementing polygenic risk scores for obesity on a larger scale: Do the potential benefits of knowing your polygenic risk score outweigh the potential harmful effect of this knowledge? There is evidence that hearing about a high genetic risk of obesity can negatively affect hunger and food-related behavior, even in individuals who actually have a low genetic risk for obesity.(15) This should warn geneticists and other health professionals who perform genetic tests for obesity that the way they interpret and communicate the test results can also have an impact on the course of the disease itself.

New diagnostic tools for hereditary obesity are being developed at a fast pace. Polygenic risk models that more closely resemble the biological pathway of obesity could help to better explain the variable phenotype of our patients. Another crucial biological mechanism that will probably solve part of the puzzle of obesity susceptibility, is epigenetics. The epigenetic programming of germ cells and embryos is a crucial process for the control of growth and metabolism in humans. For this reason, almost all patients with congenital imprinting disorders show growth abnormalities or metabolic disturbances such as diabetes.(16) On the other hand, the contribution of epigenetic changes to common obesity still holds many unanswered questions, but it is clear that environmental factors can influence the epigenetic mechanisms that underlie the development of obesity.(17) The facts that these epigenetic marks appear to be modifiable holds great potential for future therapeutic interventions.

When you want to treat obesity, you have to treat society

Obesity is an umbrella term for many different conditions characterized by an unhealthy amount of body fat, ranging from medication-induced obesity to genetic obesity disorders. And although this thesis focuses on rare genetic obesity disorders, we cannot overlook the importance of lifestyle and societal factors in the development of all types of obesity. A healthier and less obesogenic environment is always a vital ingredient of successful obesity prevention and treatment, also for patients with genetic obesity disorders. The main lifestyle-related culprit for obesity in The Netherlands seems to be excessive intake of food.(18) We might even say that our society as a whole has hyperphagia. Ambitious approaches in food policy are therefore needed, combined with tackling the socioeconomic inequalities that play an important role in the current obesity epidemic.(19) Another

worldwide societal problem with regards to obesity that we need to address is weight stigma. People with obesity frequently encounter negative views and discrimination, which leads to adverse health outcomes and a high psychological burden.(20) Health care professionals and others involved in obesity research and care need to educate the general public about the complex nature of the disease obesity. Geneticists will have to participate in these efforts by providing information on the important role that genetic variation plays in the regulation of body weight and appetite.

In conclusion

The results published in this thesis have improved the knowledge of rare genetic obesity disorders. We showed that these disorders are difficult to recognize and that extensive phenotyping with growth curve analysis is needed to establish the underlying causes of obesity. Therefore, a broad diagnostic approach including sequencing of multiple genes is warranted in all patients with early-onset severe obesity and hyperphagia. Once a genetic diagnosis is established, a treatment and follow-up plan has to be made based on the clinical characteristics of that specific disorder. Treatment targeted on the underlying genetic defect is available in selected cases. Future (inter)national collaborations are needed to improve these therapeutic options and to make evidence-based treatment and surveillance protocols for rare genetic obesity disorders.

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Appendix

NEDERLANDSE SAMENVATTING

Genetische obesitas: Aandoeningen en Diagnostiek

Introductie

Obesitas is een veelvoorkomende aandoening met ernstige gevolgen voor de gezondheid en het welzijn van patiënten. Bij een klein deel van de mensen met obesitas is een verandering in het genetisch materiaal de belangrijkste oorzaak van de obesitas. Hoofdstuk 1 bevat de inleiding van dit proefschrift waarin zeldzame genetische vorm van obesitas geïntroduceerd worden. Het proefschrift is verder in drie delen verdeeld. Het eerste deel gaat over het genotype en de moleculaire diagnostiek van deze aandoeningen. Het tweede deel is gericht op het fenotype van de patiënten. Het derde deel gaat over de impact die een dergelijke diagnose met zich meebrengt.

Deel 1 – DNA diagnostiek naar zeldzame genetische vormen van obesitas

In de afgelopen 25 jaar is er enorme vooruitgang geboekt in de diagnostiek van zeldzame genetische vormen van obesitas. Aanvankelijk waren er meerdere opeenvolgende Sanger-sequentietests nodig om een diagnose te kunnen stellen, wat een tijdrovend en kostbaar proces was. In 2012 ontwikkelde onze onderzoeksgroep een van de eerste NGS-genpanels voor genetische obesitas. Hoofdstuk 2 beschrijft de resultaten van dit genpanel voor obesitas bij 1230 kinderen, adolescenten en volwassenen met obesitas. We waren in staat om een diagnose te stellen bij ongeveer 4% van de patiënten. Dit percentage was hoger dan in eerdere studies van niet-consanguïne patiënten met obesitas. Bovendien toonden we aan dat genpanels voor genetisch obesitas een breed palet aan genen moeten omvatten, gezien we diagnoses stelden aan de hand van mutaties in 11 verschillende genen. De hoogste diagnostische opbrengst kan worden behaald in subgroepen van kinderen waarbij de obesitas op zeer jonge leeftijd begint. Hoofdstuk 3 toonde de opbrengst van dezelfde genpaneltest, die werd uitgevoerd bij 1014 patiënten die een bariatrische operatie ondergingen. In deze subgroep was de diagnostische opbrengst lager en minder heterogeen; pathogene mutaties werden slechts in vijf verschillende genen geïdentificeerd.

Deel II - Uitgebreide fenotypering om zeldzame genetische vormen van obesitas te onderscheiden van gewone obesitas

Omdat de prevalentie van obesitas hoog is en de kosten van DNA-diagnostiek hoog zijn, is het niet mogelijk om bij alle patiënten met (ernstige) obesitas ook genetische diagnostiek aan te bieden. Een beter inzicht in het klinisch fenotype van patiënten met een erfelijke vorm van obesitas is daarom nodig om te bepalen welke patiënten genetisch getest moeten worden. In hoofdstuk 4 hebben we onze systematische diagnostische benadering beschreven, die we gebruiken om de onderliggende medische oorzaken van obesitas bij kinderen te identificeren. Deze benadering omvat niet alleen genetische oorzaken van obesitas, maar ook endocriene, cerebrale en medicatie-geïnduceerde obesitas. We toonden

aan dat deze systematische beoordeling van het fenotype ons hielp om de diagnostische opbrengst te verhogen. We identificeerden een onderliggende oorzaak bij 19% van de kinderen met ernstige obesitas. Bovendien waren we in staat om enkele klinische indicatoren voor genetische vormen van obesitas te identificeren. In hoofdstuk 5 hebben we een overzicht gegeven van het fenotype van een specifieke genetische vorm van obesitas, leptinereceptordeficiëntie (LRD). Vanwege de grote variabiliteit van de aandoening, formuleerden we de hypothese dat LRD een ondergediagnosticeerde aandoening is. Op basis van gegevens van de dragerschapsfrequentie van 77165 Europese individuen, berekenden we dat LRD veel vaker voorkomt dan momenteel bekend is. Aangezien er momenteel meerdere klinische onderzoeken met nieuwe medicamenteuze therapie voor deze aandoening worden uitgevoerd, is het diagnosticeren van LRD belangrijker dan ooit.

Deel III - Het belang van een diagnose bij zeldzame genetische vormen van obesitas

In deel 3 tonen we het belang aan van de diagnoses die in de vorige twee delen zijn beschreven. In hoofdstuk 6 presenteren we vier casusbeschrijvingen over verschillende genetische obesitasaandoeningen. Het eerste artikel beschrijft een meisje met ernstige obesitas en hyperfagie, waarbij de genetische diagnose van LRD een evidente verandering in BMI veroorzaakte. In het tweede artikel van hoofdstuk 6 wordt een meisje met het Schaaf-Yang syndroom beschreven dat op de leeftijd van 23 maanden overleed. We stellen dat een strikte controle en behandeling van obesitas bij patiënten met het Schaaf-Yang-syndroom nodig is, aangezien obesitas en de syndroomgerelateerde longproblemen, hypotonie en apneu een fatale combinatie kunnen zijn. In het derde artikel rapporteerden we een geval van PTEN hamartoomtumorsyndroom, wat laat zien dat genetische analyse in de bariatrische kliniek belangrijke gevolgen kan hebben. In ons laatste case report in hoofdstuk 6 presenteren we een 11-jarig meisje met de klinische diagnose Bardet-Biedl-syndroom. We identificeerden biallelische varianten in het *IFT74*-gen, een gen dat slechts één keer eerder werd gerapporteerd als mogelijk betrokken bij het Bardet-Biedl syndroom. Op basis van deze bevinding adviseren wij om *IFT74* toe te voegen aan genpanels voor Bardet-Biedl-syndroom en ciliopathieën. We sluiten deel 3 af met een kwalitatieve studie in hoofdstuk 7, waarin de ervaringen en perspectieven werden beschreven van ouders van kinderen met een 16p11.2 deletie, één van de syndromale vormen van genetische obesitas beschreven in dit proefschrift.

Conclusie

Hoofdstuk 8 en 9 omvatten de discussie van het proefschrift en de Engelse samenvatting. De resultaten die in dit proefschrift zijn beschreven, hebben geholpen om de kennis van zeldzame genetische vormen van obesitas te vergroten. We toonden aan dat deze aandoeningen moeilijk te herkennen zijn en dat uitgebreide fenotypering met groeicurve-analyse nodig is om de onderliggende oorzaken van obesitas vast te stellen. Hierom is een

brede diagnostische benadering, inclusief sequencing van meerdere genen, gerechtvaardigd bij alle patiënten met early-onset ernstige obesitas (onder de leeftijd van 5 jaar) en hyperfagie. Zodra een genetische diagnose is gesteld, moet een behandelings- en monitoringsplan worden gemaakt op basis van de klinische kenmerken van die specifieke aandoening. Behandeling gericht op het onderliggende genetische defect is op dit moment maar in enkele gevallen beschikbaar. Toekomstige internationale samenwerking is nodig om deze therapeutische opties te verbeteren en om evidence-based protocollen te ontwikkelen voor deze zeldzame genetische vormen van obesitas.

LIST OF PUBLICATIONS

Publications in this thesis

Genetic obesity: next-generation sequencing results of 1230 patients with obesity. **Kleinendorst L**, Massink MPG, Cooiman MI, Savas M, van der Baan-Slootweg OH, Roelants RJ, et al. J Med Genet. 2018;55(9):578-86.

Obesity and Bariatric Surgery Outcome in 1014 Patients with Morbid Obesity. Cooiman MI, **Kleinendorst L**, Aarts EO, Janssen IMC, van Amstel HKP, Blakemore AI, et al. Genetic Obes Surg. 2020;30(2):470-7.

Identifying underlying medical causes of pediatric obesity: Results of a systematic diagnostic approach in a pediatric obesity center. **Kleinendorst L***, Abawi O, van der Voorn B, Jongejan M, Brandsma AE, Visser JA, et al. Plos One. 2020;15(5):e0232990. * Both authors contributed equally to this article.

Leptin receptor deficiency: a systematic literature review and prevalence estimation based on population genetics. **Kleinendorst L***, Abawi O, van der Kamp HJ, Alders M, Meijers-Heijboer HEJ, van Rossum EFC, et al. Eur J Endocrinol. 2020;182(1):47-56. * Both authors contributed equally to this article.

Young girl with severe early-onset obesity and hyperphagia. **Kleinendorst L**, van Haelst MM, van den Akker ELT. BMJ Case Rep. 2017.

The role of obesity in the fatal outcome of Schaaf-Yang syndrome: Early onset morbid obesity in a patient with a MAGEL2 mutation. **Kleinendorst L**, Pi Castan G, Caro-Llopis A, Boon EMJ, van Haelst MM. Am J Med Genet A. 2018;176(11):2456-9.

Genetic analysis in the bariatric clinic; impact of a PTEN gene mutation. Cooiman MI, **Kleinendorst L**, van der Zwaag B, Janssen IMC, Berends FJ, van Haelst MM. Mol Genet Genomic Med. 2019;7(6):e00632.

Second case of Bardet-Biedl syndrome caused by biallelic variants in IFT74. **Kleinendorst L**, Alsters SIM, Abawi O, Waisfisz Q, Boon EMJ, van den Akker ELT, et al. Eur J Hum Genet. 2020;28(7):943-6.

Who ever heard of 16p11.2 deletion syndrome? Parents' perspectives on a susceptibility copy number variation syndrome. **Kleinendorst L**, van den Heuvel LM, Henneman L, van Haelst MM. Eur J Hum Genet. 2020;28(9):1196-204.

Book chapters

Genetics of Obesity. **Kleinendorst L**, van Haelst MM, van den Akker ELT. Exp Suppl. 2019;111:419-41.

Molecular basis of obesity disorders. **Kleinendorst L**, van Haelst MM. In: Clinical Molecular Medicine. Kumar D, editor. Academic Press; 2020. p. 73-88.

Other publications

DLG4-related synaptopathy: a new rare brain disorder. Rodríguez-Palmero A, Boerrigter MM, Gómez-Andrés D, Aldinger KA, Marcos-Alcalde Í, Popp B, Everman DB, Lovgren AK, Arpin S, Bahrambeigi V, Beunders G, Bisgaard AM, Bjerregaard VA, Bruel AL, Challman TD, Cogné B, Coubes C, de Man SA, Denommé-Pichon AS, Dye TJ, Elmslie F, Feuk L, García-Miñaúr S, Gertler T, Giorgio E, Gruchy N, Haack TB, Haldeman-Englert CR, Haukanes BI, Hoyer J, Hurst ACE, Isidor B, Soller MJ, Kushary S, Kvarnung M, Landau YE, Leppig KA, Lindstrand A, **Kleinendorst L**, MacKenzie A, Mandrile G, Mendelsohn BA, Moghadasi S, Morton JE, Moutton S, Müller AJ, O'Leary M, Pacio-Míguez M, Palomares-Bralo M, Parikh S, Pfundt R, Pode-Shakked B, Rauch A, Repnikova E, Revah-Politi A, Ross MJ, Ruivenkamp CAL, Sarrazin E, Savatt JM, Schlüter A, Schönewolf-Greulich B, Shad Z, Shaw-Smith C, Shieh JT, Shohat M, Spranger S, Thiese H, Mau-Them FT, van Bon B, van de Burgt I, van de Laar IMBH, van Drie E, van Haelst MM, van Ravenswaaij-Arts CM, Verdura E, Vitobello A, Waldmüller S, Whiting S, Zweier C, Prada CE, de Vries BBA, Dobyns WB, Reiter SF, Gómez-Puertas P, Pujol A, Tümer Z. Genet Med. 2021.

Bariatric Surgery for Monogenic Non-syndromic and Syndromic Obesity Disorders. Vos N, Oussaada SM, Cooiman MI, **Kleinendorst L**, Ter Horst KW, Hazebroek EJ, et al. Curr Diab Rep. 2020;20(9):44.

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Extensive Phenotyping for Potential Weight-Inducing Factors in an Outpatient Population with Obesity. Savas M, Wester VL, Visser JA, **Kleinendorst L**, van der Zwaag B, van Haelst MM, et al. Obes Facts. 2019;12(4):369-84.

The pathogenesis of obesity. Oussaada SM, van Galen KA, Cooiman MI, **Kleinendorst L**, Hazebroek EJ, van Haelst MM, et al. Metabolism. 2019;92:26-36.
[A girl with 16p11.2 deletion syndrome]. **Kleinendorst L**, Sno M, van Haelst MM. Ned Tijdschr Geneeskd. 2019;163.

[Genetic obesity: new diagnostic options]. de Vries TI, Alsters SI, **Kleinendorst L**, van Haaften G, van der Zwaag B, van Haelst MM. Ned Tijdschr Geneeskd. 2017;161:D688.

AUTHOR CONTRIBUTIONS

- **Genetic obesity: next-generation sequencing results of 1230 patients with obesity**
  L. Kleinendorst, M.P.G. Massink, M.I. Cooiman, Mesut Savas, O.H. van der Baan-Slootweg, R.J. Roelants, I.C.M. Janssen, H.J. Meijers-Heijboer, N.V.A.M. Knoers, J.K. Ploos van Amstel, E.F.C. van Rossum, E.L.T. van den Akker, G. van Haaften, B. van der Zwaag, M.M. van Haelst. Contributors LK, MM, GvH, BvdZ and MMvH contributed to the study design and acquisition and/or analysis of the data. LK: writing and figures. All authors contributed to the data interpretation, provided critical revisions of the manuscript and approved the final version.

- **Obesity and Bariatric Surgery Outcome in 1014 Patients with Morbid Obesity**
  M.I. Cooiman, L. Kleinendorst, E.O. Aarts, I.M.C. Janssen, J.K. Ploos van Amstel, A.I. Blakemore, E.J. Hazebroek, H.J. Meijers-Heijboer, B. van der Zwaag, F.J. Berends, M.M. van Haelst. Contributor MIC was first author of this article. Contributors MIC, LK, BvdZ and MMvH contributed to the acquisition and/or analysis of the data. Literature search was performed by MC and LK. All authors contributed to the data interpretation, provided critical revisions of the manuscript and approved the final version.

- **Identifying underlying medical causes of pediatric obesity: Results of a systematic diagnostic approach in a pediatric obesity center**
  L. Kleinendorst, O. Abawi, B. van der Voorn, M.H.T.M. Jongejan, A.E. Brandsma, J.A. Visser, E.F.C. van Rossum, B. van der Zwaag, M. Alders, E.M.J. Boon, M.M. van Haelst, E.L.T. van den Akker. Contributors LK and OA contributed equally to this article. Literature search was performed by LK, OA, BvdV, BvdZ, MA, EMJB, MMvH, ELTvdA; study design by all authors except MA; data collection by LK, OA, HTMJ, AEB, BvdZ, EMJB, MMvH, ELTvdA; data analysis by LK, OA, BvdZ, MA, EMJB; data interpretation by all authors except HTMJ; generation of figures by LK, OA; writing by LK, OA, MMvH, ELTvdA; critical revision for important intellectual content by all authors.

- **Leptin receptor deficiency: a systematic literature review and prevalence estimation based on population genetics**
  L. Kleinendorst, O. Abawi, H.J. van der Kamp, M. Alders, H.E.J. Meijers-Heijboer, E.F.C. van Rossum, E.L.T. van den Akker, M.M. van Haelst.
  Contributors LK and OA contributed equally to this article. Literature search was performed by LK and OA. LK, OA, HJvdK, MA, HEJM-H, EFCvR, ELTvdA, and MMvH contributed to acquisition and/or analysis or interpretation of the data. Writing

and generation of figures by LK and OA. Critical revision for important intellectual content by all authors.

- **Young girl with severe early-onset obesity and hyperphagia**
  L. Kleinendorst, M.M. van Haelst, E.L.T. van den Akker. BMJ Case Rep. 2017. LK: main author, drafted the case report, collected the data of the patient, worked on the interpretation of the test results, communicated with the parents about the case report and searched for and selected the relevant literature and guidelines. MMvH: clinical genetics counselling of the patient and parents, designed and developed the genetic test, interpretation of the genetic test result, language editing and revising of the work for important intellectual content. ELTvdA: cared for the patient, collected data about the patient, interpretations of the endocrine lab results, assistance selecting literature and guidelines, writing assistance and revising of the work for important intellectual content.

- **The role of obesity in the fatal outcome of Schaaf-Yang syndrome: Early onset morbid obesity in a patient with a MAGEL2 mutation.**
  L. Kleinendorst, G. Pi Castan, A. Caro-Llopis, E.M.J. Boon, M.M. van Haelst. LK: main author, drafted the case report, worked on the interpretation of the data and test results, searched for and selected the relevant literature and guidelines. LK, GPC, AC-L, EMJB: data acquisition/analysis/interpretation. MMvH: interpretation of the genetic test result, language editing and revising of the work for important intellectual content.

- **Genetic analysis in the bariatric clinic; impact of a PTEN gene mutation**
  M.I. Cooiman, L. Kleinendorst, B. van der Zwaag, I.M.C. Janssen, F.J. Berends FJ, M.M. van Haelst. MIC: main author, drafted the case report. Literature search by MIC and LK. All authors contributed to the data interpretation, provided critical revisions of the manuscript and approved the final version.

- **Second case of Bardet-Biedl syndrome caused by biallelic variants in IFT74**
  L. Kleinendorst, S.I.M. Alsters, O. Abawi, Q. Waisfisz, E.M.J. Boon, E.L.T. van den Akker, M.M. van Haelst. LK: main author, drafted the case report. Data acquisition and analysis: LK, SIMA, OA, QW, EMJB. All authors contributed to the data interpretation, provided critical revisions of the manuscript and approved the final version.

- **Who ever heard of 16p11.2 deletion syndrome? Parents' perspectives on a susceptibility copy number variation syndrome**
  L. Kleinendorst, L.M. van den Heuvel, L. Henneman, M.M. van Haelst. Contributors LK and LMvdH contributed to the study design, performed the focus group interviews, and contributed to acquisition and analysis of the data. LK: writing, literature search. All authors contributed to the data interpretation, provided critical revisions of the manuscript and approved the final version.

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PHD PORTFOLIO

1. PhD Training		
	Year	**Workload (ECTS)**
General courses		
Course Castor database	2018	0.1
Basic course in Legislation and Organisation for Clinical Researchers (BROK)	2018	1.0
Intervision Tutorship VUmc	2017	0.1
Teachers training VUmc	2017	0.25
Specific courses		
Webinar polygenic risk scores	2020	0.1
Webinar obesity and COVID19 in children	2020	0.1
Webinar obesity and COVID19 in adults	2020	0.1
Seminars, workshops and master classes		
Symposium 'Promoveren zonder stress' AMC	2017	0.1
Workshop Health Care Financing	2017	0.1
Science Meetings Clinical Genetics AMC & VUmc	2019, 2017, 2016	0.3
Medical Business Masterclass	2017	0.5
Lustrum Symposium VKGN (Dutch Clinical Genetics Society)	2017	0.1
Symposium 'Behandeling van kinderobesitas: dik voor elkaar?' Heideheuvel	2017	0.1
Presentations		
<u>Oral presentations</u>		
Identifying underlying medical causes of pediatric obesity. Researchmeeting COACH (Centre for Overweight Adolescent and Children's Healthcare) Maastricht UMC	2020	0.5
International Obesity Genetics Network Meeting Amsterdam UMC	2020, 2019, 2018, 2017	2
Meet the expert: genetische obesitas. Information evening for parents and children with genetic obesity, Centrum Gezond Gewicht (CGG) Erasmus MC-Sophia	2020	0.25
Prevalence estimation of leptin receptor deficiency based on European allele frequencies		
- NASO spring meeting (Netherlands Association for the Study of Obesity)	2019	0.5
- Dutch Endocrine Meeting	2019	0.5

The results of extensive phenotyping and genotyping of patients with obesity visiting a specialized pediatric obesity clinic.		
- CGG Research meeting, Erasmus	2018	0.1
- NVHG meeting (Dutch Society for Human genetics)	2018	0.5
- NASO spring meeting (Netherlands Association for the Study of Obesity)	2018	0.5
Genetic Obesity in Pediatrics: What's New? Jonge Onderzoekersdag Erfelijke en Aangeboren Aandoeningen	2018	0.5
Genetic obesity and the Dutch 16p11.2 deletion/duplication syndrome cohort. Center for Integrative Genomics, Lausanne, Switzerland	2018	0.5
"Klinische genetica en erfelijke syndromen in de psychiatrie, met speciale aandacht voor het 16p11.2 deletie-/duplicatie-syndroom" Kinderpsychiatrie AMC	2018	0.1
Diagnostics of Syndromal and Monogenic Obesity		
- Wetenschapsbijeenkomst CGG Maasstad ziekenhuis	2017	0.5
- Dutch Endocrine Meeting	2017	0.5
- CGG Researchmeeting, Erasmus	2017	0.1
- Assistenten-LOG (Dutch Clinical Genetics Society)	2016	0.5
Science Meetings Clinical Genetics AMC & VUmc	2019, 2016	1
<u>Poster presentations</u>		
European Congress on Obesity	2020	0.5
European Society for Paediatric Endocrinology	2019	0.5
European Society of Human Genetics	2018	0.5
European Congress on Obesity	2018	0.5
Joint Meeting (UK and Dutch Clinical Genetics Societies meeting)	2018	0.5
(Inter)national conferences		
European Congress on Obesity, Dublin, Ireland (digital)	2020	1.0
European Association for the Study of Obesity (EASO) Collaborating Centers for Obesity Management Meeting, Gdansk, Poland	2019	0.5
European Society for Paediatric Endocrinology, Vienna, Austria	2019	1.0
European Society of Human Genetics, Milan, Italy	2018	1.0
European Congress on Obesity, Vienna, Austria	2018	1.0
Dutch Endocrine Meeting	2019, 2017	1.0
NVHG conference (Dutch Society for Human genetics)	2018, 2017	1.0

Other		
Consultation Young Dutch Health Council (Jonge Gezondheidsraad) about e-health	2019	0.25
Work visit to Center for Integrative Genomics, University of Lausanne, Switzerland	2018	0.25
Research visit to Aruba (1 month)	2018	-
Coauthor patient information website ikhebdat.nl, for children with 16p11.2 deletions/duplications	2018	0.25
Reports for patient magazine ZeldZaam about the 16p11.2 microdeletion/-duplication information evenings	2018, 2017	0.25
Coauthor booklet about Bardet-Biedl syndrome for Dutch general practitioners	2019	0.25
<u>Organization of meetings</u>		
16p11.2 Deletion/Duplication Patient meetings AMC	2019, 2018, 2017	0.75
International Obesity Genetics Network Meeting AMC	2019, 2018	0.5
Monthly Obesity Genetics Research Meeting AMC	2017-2018	0.25
<u>Patient care</u>		
Outpatient clinics for obesity genetics at		
- Centrum gezond gewicht, Erasmus MC-Sophia, department of pediatric endocrinology (4x/year)	2016-2020	
- Centrum gezond gewicht, Erasmus MC, department of internal medicine (4x/year)	2016-2020	
- Slotervaart ziekenhuis / VUmc locatie Louwesweg, department of paediatrics (4-8x/year)	2016-2017	
- Vitalys, center for bariatric surgery (4x/year)	2016-2019	
Outpatient clinic for 16p11.2 deletion/duplication		
Amsterdam UMC, location AMC (4-10x/year)	2016-2020	

2. Teaching		
	Year	**Workload (Hours/ECTS)**
Lecturing		
- Course clinical genetics for general practitioners. Nascholing Klinische Genetica voor Stichting Wham, Waarnemende Huisartsen Amsterdam	2018	0.25
- Training obesity genetics, department of physical therapy, ErasmusMC	2018	0.1
- Training obesity genetics for residents of the department of pediatrics, AMC and VUmc	2017	0.25
- Training Dutch clinical genetics residents: obesity genetics. Landelijk cursorisch onderwijs AIOS Klinische Genetica	2016	0.25
- Dysmorphology study group Bachelor Medicine (year 2) VUmc	2016	0.1
- Training clinical genetics for paramedics, 's Heeren Loo	2016	0.25
Tutoring, Mentoring		
- Substitute tutor study groups Bachelor Medicine (year 3) VUmc	2019	0.1
- Tutorship Bachelor Medicine (year 3) VUmc. *Weekly tutor meeting and study group* *Tutor counseling and guidance professional skills*	2017	2
Supervising		
- Supervision research student (Medicine)	2021	0.5
- Supervision master thesis (Medicine)	2017	1

2. Parameters of Esteem	
	Year
Grants	
- Grant Stichting Simonsfonds	2019
- KNAW (The Royal Netherlands Academy of Arts and Sciences) Ter Meulen Grant	2018
- Amsterdam Reproduction & Development Travel Grant	2018
- NASO (Netherlands Association for the Study of Obesity) travel grant	2018
Awards and Prizes	
- NASO Publication Prize (2019): Prize for the best obesity related publication of 2018	2019
- First prize poster presentation Joint Meeting (UK and Dutch Clinical Genetics Societies meeting)	2018
- Second prize oral abstract presentation Assistenten-LOG VKGN (Dutch Clinical Genetics Society)	2016

1. Television interview about our article "Identifying underlying medical causes of pediatric obesity: Results of a systematic diagnostic approach in a pediatric obesity center"

Jeugdjournaal 19-5-2020

"1 op de 5 kinderen met ernstige obesitas kan daar niks aan doen. Dat blijkt uit een onderzoek. Bij deze kinderen is het dus niet zo dat ze ongezond leven en daarom te dik zijn. Hun ernstige obesitas heeft een andere oorzaak."

2. Dutch news articles about "Identifying underlying medical causes of pediatric obesity: Results of a systematic diagnostic approach in a pediatric obesity center"

- Ouders krijgen te horen: eet dan ook gezond
  19-5-2020 AD/Algemeen Dagblad, Hanneke Van Houwelingen
- Medische oorzaak obesitas bij kinderen vaak niet gezien
  20-5-2020 Trouw
- Bij 1 op de 5 kinderen met ernstige obesitas is oorzaak medisch; Onderzoekers Amsterdam UMC en Erasmus MC
  19-5-2020 Telegraaf.nl
- Ongezond leven blijkt minder vaak oorzaak ernstige obesitas bij kinderen
  19-5-2020 nos.nl
- Obesitas bij kinderen heeft vaker medische oorzaak dan gedacht
  19-5-2020 nu.nl
- Medische verklaring obesitas bij beperkte groep kinderen
  15-6-2020 medischcontact.nl, Henk Maassen

3. German news article about our case report "Young girl with severe early-onset obesity and hyperphagia"

- Zwei Jahre alt, 30 Kilo schwer
  24-2-2019 Spiegel.de, Nina Weber

Dutch non peer-reviewed articles

Een meisje met ernstige vroeg ontstane obesitas en hyperfagie
Lotte Kleinendorst, Erica LT van den Akker, Mieke M van Haelst
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Iedere zorgprofessional kan een obese patiënt herkennen, maar niet iedereen weet wanneer genetisch onderzoek naar de oorzaak van de obesitas nodig is. De volgende patiëntencasus kan hier meer inzicht in geven. Daarnaast laat dit patiëntenverhaal goed zien wat voor impact genetische obesitas heeft op het leven van de patiënt en haar ouders. Een uitgebreid case report over Megan is recent gepubliceerd in BMJ Case Reports onder de titel "Young girl with severe early-onset obesity and hyperphagia".

Onstilbare honger
Megan is het eerste kind van gezonde ouders. Vanaf de eerste weken na haar geboorte valt op dat ze vaak huilt en veel behoefte heeft aan drinken. Ze groeit snel, zowel in gewicht als in lengte. Regelmatig heeft ze heftige driftbuien, die alleen te stoppen zijn met aanbieden van voeding. Op de leeftijd van 6 maanden wordt zij door de kinderarts naar een diëtist verwezen in verband met ernstige obesitas. De diëtist adviseert voedingsrestrictie. Desondanks blijft het meisje fors in gewicht aankomen. Op de leeftijd van 1 jaar en 9 maanden weegt ze 30 kilogram (+7,9 standaarddeviaties boven het gemiddelde voor vrouwelijke leeftijdsgenootjes). Vanwege de combinatie van vroege ernstige gewichtstoename en onverzadigbare eetlust (hyperfagie), wordt ze verwezen naar het Erasmus MC Centrum Gezond Gewicht, met de verdenking op een onderliggende aandoening.

Megan is de enige in haar familie met vroeg optredende obesitas en hyperfagie. Haar vader is als kind stevig geweest en werd slanker na de groeispurt; hij heeft nu overgewicht. Moeder is slank geweest tot aan haar eerste zwangerschap. Een jonger zusje heeft een normaal gewicht. Bij diagnostiek naar onderliggende hormonale ziekten worden hypothyreoïdie (laag schildklierhormoon) en hypercortisolisme (hoog stresshormoon) uitgesloten. Bij diagnostiek naar comorbiditeit worden geen aanwijzingen gevonden voor diabetes mellitus of vitamine D-deficiëntie. Er is wel sprake van insulineresistentie en

dyslipidemie (afwijkingen in de vetstofwisseling). Megans basaal energieverbruik is 24% lager dan normaal voor kinderen met haar gewicht.

Diagnostiek

Vanwege de ernstige obesitas op zeer jonge leeftijd in combinatie met de versterkte eetlust en onverzadigbaarheid wordt gedacht aan een monogenetische vorm van obesitas (obesitas veroorzaakt door een mutatie in één gen). Hiervoor was aanvankelijk alleen genetisch onderzoek van het *MC4R*-gen mogelijk, de meest voorkomende soort van monogenetische obesitas. In dit gen worden bij Megan geen afwijkingen gevonden. Later wordt de genetische diagnostiek uitgebreid met het obesitas-genpanel, waarbij meerdere genen die geassocieerd zijn met obesitas tegelijkertijd onderzocht worden. Hiermee worden bij Megan twee verschillende (compound heterozygote) mutaties in het *LEPR*-gen aangetoond. Hiervan is bekend dat deze onder andere resulteren in een onverzadigbare eetlust en een trager metabolisme. Beide ouders van het meisje blijken gezonde dragers te zijn van één van de mutaties. Leptinereceptor-deficiëntie is dus verklarend voor het klinische beeld bij Megan.

Leptine en leptinereceptor-deficiëntie

Vetcellen scheiden het hormoon leptine uit. De hoeveelheid leptine in het bloed stijgt naarmate vetcellen groter worden. Normaal gesproken leidt een toename van leptine tot een chemisch signaal dat honger kan stillen en een verzadigingsgevoel geeft. Bij leptinereceptor-deficiëntie blijft dit signaal uit. Dit leidt tot ernstige obesitas op zeer jonge leeftijd.(1)

Beloop

De ouders van Megan zijn opgelucht dat er een verklaring is gevonden voor het beeld van hun dochter. Zij hebben veel negatieve reacties en vooroordelen uit de omgeving moeten incasseren. Door de diagnose krijgen zij meer begrip en steun. Medicatie is er (nog) niet voor deze vorm van obesitas. Ouders krijgen orthopedagogische adviezen over het om kunnen gaan met de onverzadigbaarheid van hun dochter. Megan krijgt een aangepaste kinderwagen en schoenen. Het stellen van de diagnose heeft belangrijke invloed gehad op het gewicht. De BMI toonde eerst een extreem stijgende lijn en bereikte een maximum van 38,7 kg/m^2 (+8,7SD) op de leeftijd van bijna 2 jaar, toen de diagnose werd gesteld. Daarna daalde de BMI binnen 4 maanden naar 30 kg/m^2 (+6 SD).




Beschouwing

Bij monogenetische obesitas is de vroegtijdig optredende gewichtstoename het hoofdsymptoom. De diagnose 'monogenetische obesitas' werd afgelopen jaren maar weinig gesteld, mede doordat diagnostiek naar de betrokken genen niet breed beschikbaar was. De prevalentiecijfers van monogenetische obesitas in Nederland zijn nog niet bekend.

De volgende kenmerken kunnen richting geven wanneer genetisch onderzoek naar monogenetische obesitas aangewezen is: een ontstaansleeftijd voor 5 jaar, BMI boven de 50 of zeer therapieresistente obesitas.(2) Daarnaast is de familieanamnese belangrijk. Als er binnen families grote verschillen zijn in gewicht en eetlust, is de kans op een genetisch verschil tussen de familieleden groter. Er is ook een groep patiënten met obesitas die vanwege een ontwikkelingsachterstand of aangeboren orgaanafwijkingen in beeld komen bij de kinderarts. Andere kenmerken van deze 'syndromale obesitas' zijn een afwijkende hoofdomvang, een verminderde visus en nierafwijkingen. Voor beide groepen patiënten wordt genetisch onderzoek wordt geadviseerd in de richtlijn van de Endocrine Society.(3)

In het verleden was de diagnostiek bij een verdenking op monogenetische obesitas tijdrovend en kostbaar omdat de genen die geassocieerd zijn met obesitas stuk voor stuk onderzocht moesten worden. Nieuw in de diagnostiek van de genetische obesitas is het obesitas-genpanel van het VUmc waarin een vijftigtal genen tegelijkertijd worden getest.

Behandeling genetische obesitas

Voor sommige vormen van genetische obesitas worden nu klinische studies met medicatie verricht, voor vrijwel alle andere vormen is een multidisciplinaire aanpak vereist. Dit begint bij uitleg en informatie aan ouders en omgeving. Ouders krijgen daarnaast orthopedagogische ondersteuning voor het grenzen stellen rondom eten. Ook kunnen gespecialiseerde kinderdiëtisten op basis van calorimetrie het basale energieverbruik berekenen en de caloriebehoefte inschatten. Revalidatiearts, ergotherapeut en

kinderfysiotherapeut werken samen om de mobiliteit te bevorderen. Uiteraard is ook gezonde voeding en voldoende beweging essentieel bij de behandeling van monogenetisch veroorzaakte obesitas.

Conclusie

Deze casusbeschrijving illustreert het belang van het diagnosticeren van een onderliggende genetische oorzaak van obesitas, zelfs als hiervoor geen medicamenteuze behandeling bestaat.

Omdat genetische obesitas door veel verschillende genen kan worden veroorzaakt is er een test ontwikkeld waarbij een vijftigtal genen tegelijkertijd onderzocht worden. Om overdiagnostiek te voorkomen wordt dit onderzoek alleen geadviseerd aan patiënten met een hoge verdenking op genetische obesitas. Hopelijk is er in de toekomst medicamenteuze behandeling mogelijk specifiek gericht op het onderliggende genetisch defect.

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Toegelicht: Hyperfagie

Lotte Kleinendorst, Mieke M. van Haelst
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Wat verstaan we onder hyperfagie? Wat is de genetische basis van hyperfagie?

Te veel honger

Hyperfagie is de medische term voor overmatige eetlust en onverzadigbaarheid waardoor iemand te veel gaat eten. Hyperfagie is een belangrijk symptoom van enkele erfelijke aandoeningen die gepaard gaan met obesitas. Het is belangrijk om het onderscheid te maken met 'excessief eten' omdat dit eerder een gedragsmatig probleem is, dat gezien wordt bij eetstoornissen als bulimia nervosa en binge eating disorder.

Heeft mijn patiënt hyperfagie?

Er is helaas nog geen bewezen methode om hyperfagie betrouwbaar te kwantificeren. Meestal wordt er gebruik gemaakt van vragenlijsten. Hierin wordt bijvoorbeeld gevraagd naar stiekem eten, nachtelijk eten en het eten van ongewone zaken zoals voedsel uit de vuilnisbak of ongekookte pasta.

Om in te schatten of iemand hyperfagie heeft is het ook belangrijk om uit te vragen hoe vaak de patiënt honger heeft en hoe veel hij of zij aan eten denkt.

Het ontstaan van hyperfagie

Hongergevoel wordt gereguleerd in de hersenen, met name in de hypothalamus. Hier komen signalen binnen vanuit de maag, de darmen, de alvleesklier en het vetweefsel die kunnen zorgen voor een anorexigeen (eetlustremmend) of orexigeen (eetluststimulerend) effect. Deze signalen worden verwerkt in de leptine-melanocortine-signaalroute. Een voorbeeld van een dergelijk signaal is het hormoon leptine. Als de hoeveelheid vet in het lichaam toeneemt, wordt er ook meer leptine uitgescheiden door het vetweefsel. Leptine remt vervolgens de neuronen die orexigene signalen uitzenden. Hiermee wordt het hongergevoel dus gedempt. Bij voldoende vetweefsel is extra voedselinname immers niet noodzakelijk. Bij patiënten die geen leptine kunnen maken of geen werkzame leptinereceptoren hebben, werkt deze 'honger-stop' niet goed. Hierdoor krijgen zij hyperfagie en obesitas op zeer jonge leeftijd. Afwijkingen (mutaties) in genen die een belangrijke rol spelen in deze leptine-melanocortine-signaalroute leiden vaak op jonge leeftijd al tot obesitas . De meest voorkomende vorm van erfelijke obesitas wordt veroorzaakt door mutaties in het melanocortine 4-receptor-gen (*MC4R*).

Voorbeelden
Enkele voorbeelden van aandoeningen waarbij hyperfagie voorkomt:

Genetisch

- Monogenetische obesitas: obesitas veroorzaakt door een verandering (mutatie) in een gen, zoals bij patienten met mutaties in de genen van de leptine-melanocortine-signaalroute (bijvoorbeeld MC4R of LEPR, een leptinereceptor)
- Prader-Willi syndroom: syndroom waarbij patienten naast hyperfagie en obesitas ook een verstandelijke beperking en andere fysieke problemen hebben (een vorm van syndromale obesitas)

Niet-genetisch

- Hypothalame obesitas: obesitas bij patiënten met bijvoorbeeld een craniofaryngeoom (een zeldzame hypofysetumor)

ABOUT THE AUTHOR

Lotte Kleinendorst was born on 5 September 1990 in Amsterdam, The Netherlands. In 2008, she finished secondary education at the Vossius Gymnasium in Amsterdam and started her medical studies at the University of Amsterdam (UvA). She obtained her *cum laude* propaedeutic diploma in 2009 and followed the AMC-UvA Honours Programme during medical school. In 2012, her enthusiasm for research and interest in international politics came together during her stay in Boston, where she worked at a health care policy and research organization. When she returned, she graduated *cum laude*. After obtaining her medical degree in February 2015, she worked as a resident (ANIOS) at the Department of Genetics of the UMC Utrecht. Here, Mieke van Haelst supervised her clinical work with patients suffering from genetic obesity. At the obesity genetics outpatient clinic in the Erasmus MC, she met pediatric endocrinologist Erica van den Akker and endocrinologist Liesbeth van Rossum, the founders of the Center for Healthy Weight (*Centrum Gezond Gewicht*, CGG). In August 2016, she returned to the AMC as a PhD student. She continued with the obesity genetics outpatient clinics and performed an important part of her research at CGG. In January 2020, she started her medical specialty training at the Clinical Genetics department at Amsterdam UMC, location AMC. Her ambition is to combine her training as clinical geneticist with her research activities on obesity genetics. Furthermore, she aims to play an active role in the international multidisciplinary collaborations for rare genetic obesity disorders.

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