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Cite this article as: Katja Kohlsdorf, Adriana Nunziata, Jan-Bernd Funcke, Stephanie Brandt, Julia von Schnurbein, Heike Vollbach, Belinda Lennerz, Maria Fritsch, Susanne Greber-Platzer Elke Fröhlich-Reiterer, Manuel Luedeke, Guntram Borck, Klaus-Michael Debatin, Pamela Fischer-Posovszky and Martin Wabitsch, Early Childhood BMI Trajectories in Monogenic Obesity due to Leptin –, Leptin Receptor- and Melanocortin 4 Receptor Deficiency,*International Journal of Obesity* _####################_ doi:10.1038/s41366-018-0049-6

Received 01 June 2017; accepted 22 January 2018;
Author version _####################_

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Short title: BMI trajectories in monogenic obesity

Conflict of interest: All authors have indicated they have no potential conflicts of interest to disclose.

Abstract

Objective:

To evaluate whether early childhood body mass index (BMI) is an appropriate indicator for monogenic obesity.

Methods:

A cohort of n=21 children living in Germany or Austria with monogenic obesity due to congenital leptin deficiency (group LEP, n=6), leptin receptor deficiency (group LEPR, n=6) and primarily heterozygous MC4 receptor deficiency (group MC4R, n=9) was analyzed. A control group (CTRL) was defined that consisted of n=22 obese adolescents with no mutation in the above mentioned genes. Early childhood (0-5 years) BMI trajectories were compared between the groups at selected time points.

Results:

The LEP and LEPR group showed a tremendous increase in BMI during the first 2 years of life with all patients displaying a BMI >27 kg/m² [27.2-38.4 kg/m²] and $\%BMI_{P95}$ (percentage of the 95th percentile BMI for age and sex)>140% [144.8-198.6%] at the age of 2 years and a BMI >33 kg/m² [33.3-45.9 kg/m²] and $\%BMI_{P95}$ >184% [184.1-212.6%] at the age of 5 years. The MC4R and CTRL groups had a later onset of obesity with significantly lower BMI values at both time points ($p<0.01$).

Conclusion:

As result of the investigation of early childhood BMI trajectories in this pediatric cohort with monogenic obesity we suggest that BMI values >27.0 kg/m² or $\%BMI_{p95}$>140% at the age of 2 years and BMI values >33.0 kg/m² or $\%BMI_{p95}$>184% at the age of 5 years may be useful

cut points to identify children who should undergo genetic screening for monogenic obesity due to functionally relevant mutations in the leptin gene or leptin receptor gene.

Introduction

The two opposite sensations hunger and satiation play a pivotal role in the body's energy homeostasis. Any violation of the balance between hunger and satiation results in failures in weight regulation with the clinical phenotypes of anorexia and obesity at the very extremes of the spectrum. Leptin as the essential hunger regulating hormone and the hypothalamic leptin-melanocortin signal pathway play a pivotal role in regulating food intake (1). Monogenic defects in the leptin gene or in the leptin-melanocortin pathway are associated with an early onset of severe obesity.

Among the many children with obesity (2), as many as 1-5% of those with severe obesity may have monogenic obesity (3,4). The most common form of monogenic obesity is caused by heterozygous mutations in the melanocortin 4 receptor gene (MC4R) (5-7). Other causes are homozygous mutations in the leptin gene (LEP), leptin receptor gene (LEPR) or proopiomelanocortin (POMC) gene (8-14). Distinguishing between monogenic forms of obesity and common obesity already at an early age presents a tremendous challenge for pediatricians. The question arises when to initiate further investigations including measurement of circulating leptin, functional leptin and sequencing of obesity genes (15).

The earlier in life monogenic obesity is diagnosed, the earlier patients might receive proper treatment and might be protected from further stigmatization, from developing obesity-associated comorbidities and even more important from tedious and unsuccessful alternative therapy approaches (16, 17). The diagnosis of a monogenic cause can furthermore provide relief for affected families and might stop assignments of guilt and help to understand as well as accept obesity as a chronic disease.

Little has been published about early childhood body mass index (BMI) courses in monogenic obesity (18-20). We had the unique opportunity to analyze data on early childhood weight and height development in a cohort of n=21 children with monogenic obesity at a single center.

The objectives of this study were to describe early childhood BMI trajectories in patients with leptin deficiency, leptin receptor deficiency and MC4R deficiency and to compare BMI trajectories of patients with monogenic obesity to similarly-obese children who had no such mutations. We then aimed at generating typical cut off values for BMI and BMI-SDS which might be helpful to identify patients with monogenic obesity at an early age.

Methods

Body mass index (BMI) values were calculated as weight (in kilograms) divided by the square of height (in meters). Obesity was classified as a BMI >97th percentile and severe obesity as a BMI >99.5th percentile for age and gender using German reference data (21). BMI-SDS values were calculated using the same reference values (21). In addition, the degree of obesity was expressed as percentage of the 95th percentile (%BMI$_{P95}$) of the Center of Disease Control and Prevention (CDC) BMI percentiles (2-20 years) as recommended recently in the Endocrine Society Clinical Practice Guideline for pediatric obesity (22).

BMI values from birth until the age of 5 years were analyzed in children with (study cohort) and without early onset obesity (control cohort) due to mutations in the leptin gene, the leptin receptor or the MC4 receptor gene. All children in Germany have 10 compulsory examinations from birth till to the age of 5 years (including weight and height measurements) to exclude disorders or delays in physical or mental development (German U Screenings). Results are documented in health booklets. Using these weight and height data (German U Screening) and weight and height data from medical visits in between we calculated BMI and BMI-SDS values to analyze BMI trajectories in children of the study cohort and in children of the control cohort. In addition BMI and BMI-SDS values at three selected time points

(representing three of the above mentioned compulsory examinations, German U Screening) were compared between children of the study and children of the control cohort. These three selected time points were birth (Age0: U Screening 1, immediately after birth), 2 years of age (Age2: U Screening 7, 21 to 24 months after birth,) and 5 years of age (Age5: U Screening 9, 60 to 64 months after birth).

Study cohort with monogenic obesity

This retrospective study encompasses n=21 patients with monogenic obesity due to leptin, leptin receptor or MC4 receptor deficiency. All patients presented to, and were diagnosed in, our obesity outpatient clinic from 2003-2016. Age at presentation in our obesity outpatient clinic ranged between 11 months and 16 years of age. Written informed consent was obtained from the parents. The ethics committee of the University of Ulm approved this study. The inclusion of human subjects in this study complies with the Declaration of Helsinki.

We divided the patients into three groups according to the underlying mutation (see Table 1): congenital leptin deficiency (LEP), leptin receptor deficiency (LEPR) and MC4 receptor deficiency (MC4R).

Group LEP comprised n=3 patients (2 male, 1 female) with leptin deficiency (LEP_1-3) and n=3 patients (2 male, 1 female) with biologically inactive leptin (LEP_4-6) due to homozygous mutations in the leptin gene. Clinical characteristics of 4 patients have been published earlier (11, 12, 23). As patients LEP_1, LEP_3 and LEP_4 received hormone replacement therapy before reaching the age of 5 years, we analyzed their data on height and weight development only up to the time point when therapy was started.

Group LEPR comprised n=6 patients (4 male, 2 female) with leptin receptor deficiency (LEPR_1-6) due to homozygous (n=4) or compound heterozygous (n=2) mutations in the

leptin receptor gene. Patient LEPR_2 was the only child born pre-term with a gestational age of 30+6 weeks. LEPR_2 had normal birth weight (50^{th} percentile) and birth length (25^{th}-50^{th} percentile) according to German percentile values for pre-term born children (25). Due to prematurity, the patient's data were not included in the calculation of mean BMI und BMI-SDS at Age0.

Clinical characteristics of 14 patients identified at our center with functionally relevant MC4R mutations have been published earlier(5). For 9 of these 14 patients data on early childhood weight and height was available. Group MC4R comprised these 9 patients (7 male, 2 female) with MC4R deficiency (MC4R_1-9) due to heterozygous (n=8) or compound heterozygous (n=1) mutations in the MC4R gene.

Control group with severe obesity

All patients presented to, and were diagnosed in, our obesity outpatient clinic due to severe obesity were recruited between June 2012 to November 2014. Following inclusion criteria for the control group were defined: BMI at the ages 14, 15 or 16 years >30 kg/m², early childhood data on BMI available (at least 2 values between 0-5 years) and exclusion of a mutation in the leptin-, leptin receptor and MC4R gene. The control group (CTRL) included n=22 severely obese patients. Mean age at presentation was 15.1±0.8 years.

Statistical analyses

Patients' anthropometrics were summarized by use of descriptive statistics including mean, standard deviation (SD), standard error of the mean (SEM) and standard deviation score (SDS, z-score). The data given in square brackets always represent the whole data range. Curve fitting and statistical analyses were performed using GraphPad Prism version 6.01 (GraphPad Software, La Jolla, CA, USA). Third order polynomial curves were fitted to the

BMI data using the robust fit method. One-way ANOVAs with Holm-Sidak corrections for multiple testing were used to analyze the BMI-SDS at birth (Age0), 2 years (Age2) and 5 years of age (Age5). The significance level (α) was set to be 0.05.

Results

Early childhood BMI trajectories

All patients had a normal BMI at Age0. Patients with congenital leptin deficiency or leptin receptor deficiency showed an immediate onset of rapid weight gain after birth with the most intense increase in BMI occurring during their first year of life. In all cases, the BMI in LEP and LEPR groups was >27 kg/m² at Age2 or $\%BMI_{p95}$ >140% and >33 kg/m² at Age5 or $\%BMI_{p95}$ >184%, which complied with severe obesity at both time points (21, 22). In contrast to this surveillance, the weight gain in MC4R patients and patients of the severely obese control group was less pronounced, especially over the first 2 years of life. Their BMI was clearly <27 kg/m² at Age2 and also <33 kg/m² at Age5 except for one obese control who displayed a BMI of 33.8 kg/m² at Age5. However, most importantly and in contrast to LEP and LEPR, this patient of the control had a BMI <27 kg/m² (BMI 22.5 kg/m²) at Age2. Interestingly, patient MC4R_4, who had a compound heterozygous mutation, displayed the most severe phenotype among all patients in the MC4R group. A selection of BMI data is provided in table 2. Early childhood BMI trajectories are shown in figure 1.

In summary, these data show that absolute BMI values and percentage of the 95th percentile values are comparable between patients with congenital leptin deficiency and leptin receptor deficiency but remarkably higher than those in patients with impaired MC4R or patients in the severely obese control group.

Early childhood BMI-SDS

There were no significant differences in BMI-SDS values between all groups at birth (all $p>0.2$). Comparing LEP and LEPR, BMI-SDS values also did not differ significantly at Age0, Age2 or Age5 ($p>0.5$). All patients had BMI-SDS values above 4.0 [$BMI\text{-}SDS_{min\text{-}max}$ 4.3-7.0] at Age2 and Age5. Although in average BMI-SDS values declined towards Age5, they still remained above 4.0 and were thus significantly higher compared to MC4 receptor deficiency and patients in the severely obese control group at both time points (all $p<0.01$).

In summary, our data show remarkable differences between the groups with significantly higher BMI-SDS values in LEP and LEPR compared to those in MC4 receptor deficiency and patients in the severely obese control group (Figure 2).

Discussion

By analyzing early childhood data of weight and height in a cohort of n=21 patients with monogenic obesity and a control group of children with severe obesity (n=22) we described and compared BMI trajectories, BMI values and BMI-SDS values from birth until the age of 5 years. The cohort consisted of patients with leptin deficiency due to homozygous mutations in the leptin gene, patients with leptin receptor deficiency due to homozygous or compound heterozygous mutations in the leptin receptor gene (n=1) and patients with MC4R deficiency due to functional relevant, heterozygous mutations or compound heterozygous mutations in the MC4R receptor gene. We suggest absolute BMI cut-off values as well as BMI cut-off values expressed as the percentage of the 95^{th} percentile of the CDC BMI percentiles (2-20 years) appropriate to distinguish patients with leptin deficiency and leptin receptor deficiency from those with impaired MC4R function due to heterozygous mutations in the MC4R gene as well as from children with severe obesity in whom mutations in the leptin, leptin receptor and MC4R gene were excluded.

Leptin´s main functions are to control body fat mass and to maintain energy homeostasis. To achieve this, the adipocyte-derived hormone leptin initiates a series of changes in energy intake, energy expenditure, autonomic nervous system tone and neuroendocrine functions *via* both, melanocortin-dependent and melanocortin-independent pathways (8). Concerning the leptin-melanocortin pathway, leptins interaction with its hypothalamic receptor activates proopiomelanocortin-neurons in the arcuate nucleus and induces the production of alpha-melanocyte-stimulating hormone which transmits leptin´s anorectic effect through the melanocortin 4 receptor.

Impaired leptin signaling in the central nervous system can be caused by homozygous mutations in the leptin gene resulting in a lack of hormone production, hormone secretion or biological activity due to impaired receptor binding (11, 12, 14). Moreover, homozygous or compound heterozygous mutations in the leptin receptor gene can lead to defects in receptor expression or receptor signaling. In all these conditions, the clinical phenotype is characterized by impaired satiety, hyperphagia and food seeking behavior leading to early-onset severe obesity, reduced sympathetic tone and endocrine alterations such as hypogonadism (8-12, 14, 20, 23, 25). Unfortunately, only a few monogenic forms of monogenic obesity offer the opportunity of pharmacological treatment. Hormone replacement in patients with leptin deficiency has been proven to be successful (10, 12, 23, 26). Promising results of treating patients with POMC deficiency with a MC4R agonist have been published recently and this treatment might moreover be an option for patients with leptin receptor deficiency in the future (13).

In the present study, BMI and BMI-SDS values did not differ significantly between patients with either leptin deficiency or leptin receptor deficiency. Both entities displayed close to identical BMI trajectories in early childhood. Yet, the BMI and BMI-SDS values were significantly higher and clearly distinguishable from those of patients with heterozygous

mutations in the MC4R gene and obese controls. All patients with leptin deficiency or leptin receptor deficiency presented with a BMI >27 kg/m² or $\%BMI_{p95}$ >140% at 2 years of age as well as >33 kg/m² or $\%BMI_{p95}$ >184% at 5 years of age. In contrast, BMI values of our mostly heterozygous MC4R deficiency patients and obese controls remained clearly below these cut-offs.

In our cohort, MC4R deficient patients had a later onset of obesity with significantly lower BMI and BMI-SDS values compared to leptin- or leptin receptor deficient patients. In the literature, penetrance and expressivity of the obese phenotype in MC4R deficiency varies considerably more than the extremely obese phenotype in all patients with leptin or leptin receptor deficiency (3-5, 19). This variability is explained by differences in the functional relevance of the MC4R variants and by the influence of variable obesogenic environmental factors (4, 27, 28). Carriers of homozygous mutations of the MC4 receptor gene with a complete loss of function are rare and present the severest phenotype including an earlier onset of severe obesity (4, 19, 29). The more frequent carriers of mutations with residual function or heterozygous status seem less affected (3-5). In contrast to patients with leptin deficiency or leptin receptor deficiency, a very early onset of severe obesity is not a specific clinical feature of carriers with heterozygous MC4R mutations. These findings are coherent with our results and are an explanation for the later onset of obesity in our MC4R mutation carriers since they were all in heterozygous status. Not surprisingly, the most severe phenotype was displayed by a patient who carried two heterozygous MC4R mutations in *trans*. We would like to point out that although being compound heterozygous, this patient as well as one patient of the control group who displayed the highest absolute BMI value at the age of 5 years did not reach BMI values at the age of 2 years comparable to those of patients with either leptin deficiency or leptin receptor deficiency.

A limitation of this study is that the patients of the control group have been presented for obesity evaluation to our obesity outpatient clinic later than the patients of the group with monogenic obesity. Therefore a direct control group with patients presented for obesity evaluation at the same age is missing. Further limitation presenting absolute BMI values as upper thresholds lies in the fact that these values are based on data from patients, who were growing up in Western Europe. Weight development in other populations, under different living conditions, might not be comparable. However, BMI-SDS values do not provide a satisfactory population-based normalization for extremely obese individuals. Recent investigations based on the 2000 Center for Disease Control and Prevention (CDC) growth charts have shown limitations of transforming very high BMI values into BMI-SDS values in children and suggest instead the use of BMI as a percentage of the age and sex specific 95th percentile (%BMI_{P95}) to estimate the severity of obesity and BMI development (22, 30, 31).

A wide range of very high BMI values has shown to map to similar BMI-SDS values. Furthermore, very high BMI SDS values vary by sex and age. Since German references also used the LMS method to calculate BMI-SDS values we could presume similar limitations (32). When we implemented BMI as a percentage of the 95th percentile in our analyses to estimate changes in BMI we could see an aggravation of obesity in patients with leptin or leptin receptor deficiency (2 years of age %BMI_{P95}>140%, 5 years of age %BMI_{P95}>184%) while BMI-SDS values tended to decline. Due to the shown limitations of BMI-SDS values in severely obese children we propose the use of absolute BMI values or BMI as a percentage of the 95th percentile value using CDC charts or a population specific BMI percentile if available as cut offs for the rare condition of leptin deficiency and leptin receptor deficiency.

Early onset severe obesity is a rare condition. Based on the description of typical BMI trajectories early in life, we suggest further investigations of leptin and its receptor in children who present with a BMI >27 kg/m² or %BMI_{P95}>140% at the age of 2 years and a BMI >33

kg/m² or %BMI_{P95}>184% at the age of 5 years. Our data demonstrate that early childhood BMI is a useful parameter to distinguish patients carrying a functional relevant homozygous or compound heterozygous mutation in the leptin -or leptin receptor gene from patients heterozygous mutations in the MC4 receptor gene and severe obesity in which mutations of the leptin-, leptin receptor and MC4 receptor have been excluded.

Since pharmacological treatment is already an option for patients with leptin deficiency and might be an option for patients with leptin receptor deficiency in future early detection of these rare conditions by following early childhood BMI trajectories is important. Proper diagnostic work up and treatment early in life offers the opportunity to prevent children and their families from further stigmatization as well as from the failure of conservative treatment approaches.

Funding

The study was funded by the German Ministry of Education and Research (BMBF) and is integrated in the Competence Network Obesity (FKZ 01GI1120A).

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Figures

Figure 1: BMI trajectories of patients with monogenic obesity due to mutations in the leptin gene (A), leptin receptor gene (B), MC4R gene (C) and obese controls (D). Displayed are third order polynomial curves that were fitted to the BMI data, individual patient BMI data (in the background) as well as the 3rd, 50th, 90th, 97th and 99.5th BMI percentile according to German reference date for boys given the majority of the sample were boys (21).

Figure 2: BMI-SDS values at birth, at 2 and 5 years of age of patients with monogenic obesity due to mutations in the leptin gene (LEP), leptin receptor gene (LEPR) or MC4 receptor gene (MC4R) and obese controls (CTRL). Displayed are means ± SEMs of the BMI-SDS data and individual BMI-SDS values (symbols).

Table 1: Overview of the individual mutations in our cohort of n=21 patients with monogenic obesity due to mutations in the leptin gene (LEP), leptin receptor gene (LEPR) and melanocortin 4 receptor gene (MC4R).

gene	*patient*	*mutation on cDNA level*	*mutation on protein level*
LEP	LEP1	c.313C>T	p.R105T
	LEP2	c.21T>C	p.L72S
	LEP3	c.313C>T	p.R105T
	LEP4	c.298G>T	p.D100Y
	LEP5	c.309C>A	p.N103K
	LEP6	c.309C>A	p.N103K
LEPR	LEPR1	deletion exon 4-20	n.d.
	LEPR 2	c.2051A>C	p.H684P
	LEPR 3	c.2227T>C	p.S743P
		c.2598-3_2607delTAGAATGAAAAAG	splice defect (not experimentally tested)
	LEPR 4	c.946C>	p.P316T
		c.1938G>T	p.W646C
	LEPR 5	c.1874G>A	p.W625*
		c.2051A>C	p.H684P
	LEPR 6	c.461dupA	p.N154Kfs*3
MC4R	MC4R1	c.466C>T	p.Q156*
	MC4R 2	c.542G>A	p.G181D
	MC4R 3	c.268G>A	p.D90N
	MC4R 4	c.105C>A	p.Y35*
		c.110A>T	p.D37V
	MC4R 5	c.283G>A	p.V95I
	MC4R 6	c.731C>A	p.A244E
	MC4R 7	c.124G>A	p.E42K
	MC4R 8	c.380C>T	p.S127L
	MC4R 9	c.453delC	p.F152Sfs*161

Table 2: Range, mean and standard deviation of BMI (kg/m²) and percentage of the 95th percentile of BMI based on CDC growth charts 2-20 years (%BMI$_{p95}$) in the cohort of n=21 children with monogenic obesity due to a functional relevant mutation of the leptin gene (LEP), leptin receptor gene (LEPR), mutation of the MC4 receptor gene (MC4R) and in obese controls (CLTR) at birth (Age0), and ages of 0.5, 1, 2, 4 and 5 years (Age 0.5-Age5).

group	***BMI (kg/m²)***	***Age0***	***Age0.5***	***Age1***	***Age2***	***Age4***	***Age5***
LEP	BMI_{min}	11.5	17.7	23.4	28.0	28.1	33.6
	BMI_{max}	14.9	33.7	31.2	38.4	34.4	36.0
	Mean BMI ±SD	13.1	25.0 ±5.9	27.0	31.6 ±4.6	31.2	34.8 ±1.7
	$\%BMI_{P95\ min}$	n.a.	n.a.	n.a.	144.8	155.9	184.1
	$\%BMI_{P95\ max}$	n.a.	n.a.	n.a.	198.6	190.7	200.7
	$mean\%BMI_{P95}$	n.a.	n.a.	n.a.	164.43	173.3	192.4 ±11.7
LEPR	BMI_{min}	11.1	26.97	28.10	27.22	30.82	33.37
	BMI_{max}	13.3	29.59	30.77	37.40	40.35	45.86
	Mean BMI ±SD	12.2	28.6 ±1.4	29.4	33.7 ±4.8	35.2	38.3 ±5.0
	$\%BMI_{P95\ min}$	n.a.*	n.a.	n.a.	140.8	172.8	186.0
	$\%BMI_{P95\ max}$	n.a.	n.a.	n.a.	195.2	223.8	251.2
	$mean\%BMI_{P95}$	n.a.	n.a.	n.a.	175.0	196.7	212.6 ±30.4
MC4R	BMI_{min}	11.9	16.2	15.6	15.5	16.4	18.0
	BMI_{max}	13.9	21.0	19.6	20.5	25.2	26.0
	Mean BMI ±SD	12.6	19.2 ±2.6	17.5	18.2 ±2.1	19.7	21.6 ±3.2
	$\%BMI_{P95\ min}$	n.a.	n.a.	n.a.	81.2	91.9	100.3
	$\%BMI_{P95\ max}$	n.a.	n.a.	n.a.	107.3	141.3	144.9
	$mean\%BMI_{P95}$	n.a.	n.a.	n.a.	94.4	110.0	119.6±17.8
CTLR	BMI_{min}	11.1	14.4	12.2	13.2	14.6	16.2
	BMI_{max}	16.9	19.9	21.0	22.5	30.7	33.8
	Mean BMI ±SD	13.0	17.5 ±1.5	16.9	17.4 ±2.1	19.1	19.8 ±4.0
	$\%BMI_{P95\ min}$	n.a.	n.a.	n.a.	69.4	81.0	90.1
	$\%BMI_{P95\ max}$	n.a.	n.a.	n.a.	117.5	170.2	185.0
	$mean\%BMI_{P95}$	n.a.	n.a.	n.a.	91.26	106.9	109.2 ±22.0

(n.a.; not available)