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Whole Exome Sequencing Revealed Paternal Inheritance of Obesity-related Genetic Variants in a Family with an Exclusively Breastfed Infant

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What is already known on this topic?

Obesity is a complex disorder characterized by excess body fat that manifests under the influence of genetic and environmental factors. Rapid growth during infancy and early childhood has directly been related to the onset of adult obesity. Whole exome sequencing (WES) has been used for identifying novel rare variants in disease pathogenesis. Nevertheless, the mechanism underlying this complex disease is still incompletely understood.

What this study adds?

WES analysis in combination with family segregation was used in predicting the risk for later obesity of an exclusively breastfed infant, and in providing genetic counseling to the family. The paternal inheritance of all potentially deleterious novel obesity-related variants was confirmed in the family.

Abstract

Objective: Obesity is a serious health problem that progressively affects individuals' lives with comorbidities, such as heart disease, stroke, and diabetes mellitus. Since its prevalence has increased, particularly in children less than five years old, its genetic and environmental causes should be determined for prevention and control of the disease. The aim of this study was to detect underlying genetic risk factors in a family with an exclusively breastfed obese infant.

Methods: A three-generation family was recruited to be evaluated for obesity. Detailed examinations along with body mass index (BMI) calculations were performed on available family members. Whole exome sequencing (WES) was performed on a 7-month-old obese infant. Bioinformatic analyses were performed on the Genomize SEQ platform with variant filtering at minor allele frequencies < 1 % for all normal populations. Sanger sequencing was applied in variant confirmation and family segregation.

Results: Neuro-motor developmental features were normal and genetic syndromes were excluded from the index. Early-onset severe obesity (+ 4.25 standard deviation score weight-for-height) was evident in index case; his father and grandmother were also obese (BMIs 38.1 kg/m^2 and 31.3 kg/m^2, respectively). WES analysis revealed deleterious variants in *SH2B1, PDE11A, ADCY3,* and *CAPN10* genes previously associated with obesity. All variants were evaluated as novel candidates for obesity, except *PDE11A*, and family segregation confirmed paternal inheritance.

Conclusion: This study confirmed the paternal inheritance of all potentially deleterious obesity-related variants. The cumulative effect of individual variants might explain the obesity phenotype in this family. The infant is recommended to be followed up periodically due to increased risk for later childhood obesity.

Keywords: Early-onset obesity, whole exome sequencing, paternal inheritance, novel variants, body mass index

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Introduction

Obesity is a complex disorder characterized by excess body fat that manifests through the influence of both genetic and environmental factors. Various clinical manifestations of obesity originate from diverse phenotypic expression of genetic variants (1). Polygenic/common obesity is the most prevalent type of obesity in society, which occurs because of the effect of many polymorphisms, each contributing small effects (2). There is an imbalance between energy intake and consumption, which increases adipose tissue due to a combination of genetic predisposition and environmental factors (3). However, rare abnormalities linked to a single gene may occur and are termed monogenic or Mendelian obesity. Early-onset severe obesity is the development of obesity in early life, which might occur due to disruption in genes involved in pathways affecting energy balance, appetite, and adipocyte distribution (4). Obesity increasingly affects children under the age of 5 years, highlighting the importance of the early childhood period (5). According to the World Health Organization (WHO), in 2020 nearly 39 million children under the age of 5 years were reported to be overweight or obese (6). Therefore, if protective and preventive measures are not taken, it is predicted that one-fifth of the world's population will be obese by 2025 (7).

Rapid growth during infancy, especially in the first 4 months, and in early childhood (within the first 2 years of life) have been directly linked with the onset of adult obesity (6,8). Physiologic alterations in this critical developmental period contribute to the risk of obesity and comorbidities observed in adulthood (9). Consequently, infants born large for gestational age, along with rapid early growth and physical inactivity have been identified as significant risk factors for obesity (10,11,12). Meta-analysis comprising twin, family, and adoption studies have reported that the heritability of body mass index (BMI) ranges from 40 % to 70 % , implicating the role of genetics (13,14,15). Moreover, the genetic basis has been reported to influence hyperphagia, the disruption of energy balance, and body weight regulation, which all together may lead to severe pediatric obesity (3). Hence, genetic studies involving children are important to determine the type of obesity, its diagnosis, and the risk of recurrence, as well as in providing genetic counseling (16). In this respect, genome-wide association studies (GWAS) have been used extensively to identify common, risk-conferring alleles, while whole exome sequencing (WES) was allowed the identification of novel rare variants in disease pathogenesis (17,18). GWAS exploits mostly non-coding single nucleotide polymorphisms (SNPs) to associate their impacts on the transcriptional regulation of nearby genes through comparison of cases and controls. Since 85 % of mutations are located in protein-coding regions of the genome, WES has been used for detecting deleterious rare variants in disease pathogenesis (19). Therefore, the GWAS and WES approaches complement each other in deciphering the missing heritability in complex disorders. However, in the case of obesity research, the collective efforts of these approaches have managed to explain only 2 % to 6 % of the genetic component of obesity in association with BMI variation (4,20), highlighting the need for further research in varied populations.

The aim of this study was to identify novel rare variants associated with early childhood obesity in a three-generation family having an exclusively breastfed obese infant. The inheritance of obesity appeared to be paternal. Therefore, WES analysis in combination with family segregation was used in predicting the risk for later obesity of this infant, and in providing genetic counseling to this family.

Methods

Patients and Clinical Assessments

A family from Turkiye, involving an exclusively breastfed, 7-month-old, obese male infant along with his parents and grandmother, was recruited for this study. The index (HP028) was born as the first child of non-consanguineous parents and had abnormal weight gain in the early months of life. The grandmother reported a similar growth pattern for her son, with obesity starting in his early childhood and persisting throughout his life without any comorbidity. She and her deceased spouse were reported to be obese and overweight, respectively, from early childhood. The index was subjected to a detailed clinical examination comprising physical and serological evaluations. Detailed clinical data on family history was collected from the adult recruits. In children younger than 2 years of age, obesity is diagnosed if the sex specific weight for recumbent height is more than 97.7 percentile or 2 standard deviation scores (SDS) according to WHO growth standards (21).

Weight, height, and BMI SDSs of the index case were calculated according to the growth chart prepared with national standards and weight for height SDS was based on WHO growth standards (22,23). BMI values of adult individuals were calculated by height, and weight respectively. Following WHO standards, BMI values greater than 30 kg/m^2 were defined as obese and marked with black color on the pedigree (Figure 1). Written informed consents were obtained from all individuals or legal representatives in accordance with Istanbul University, Istanbul Medical Faculty Clinical Research Ethics Committee (protocol no: 2020/1054, date: 01.09.2020). According to the manufacturer's instructions, DNA was extracted using Purelink Genomic DNA Mini Kit

from peripheral blood (Invitrogen, Thermo Fisher Scientific, Inc., Waltham, MA, USA). The quantity and purity of DNA samples were measured with NanoDrop ND2000 (Thermo Fisher Scientific Inc.) spectrophotometry and samples were run on agarose gel as a final quality control.

Whole Exome Sequencing and Familial Segregation

WES was performed in the index case under the service of Izmir Tinaztepe University, Faculty of Medicine, Medical Genetic Diagnostic Center (Izmir, Turkiye). Exonic DNA was captured with the Twist Comprehensive Exome kit (Twist Bioscience, South San Francisco, CA, USA), which was used in library preparation. 36.8 Mb of protein-coding regions covering >99% of RefSeq, CCDS, and GENCODE databases were targeted in this manner. Thereafter, sequencing was performed on the Illumina NextSeq 550 platform to achieve a minimum of 20X reading depth for the targeted bases.

Sequence annotations and variant filtering were conducted on the SEQ platform version 16.7 (https://seq.genomize.com; Genomize Inc., Istanbul, Turkiye), which processes FASTQ files by aligning to the GRCh37/hg19 reference genome with a Burrows-Wheeler Alignment (BWA) tool (24). Variants were selected with Freebayes, after duplicate products and realignments of indels were removed by Genomize's proprietary algorithms (25). Variants were annotated using VEP v102 (26). All variant prioritizations were performed for minor allele frequencies (MAF) <1% in all normal populations to detect rare variants. Initially, a whole variant list was filtered to select obesity-related genes obtained from the literature (Supplementary Table 1). Secondly, intronic and synonymous variants were filtered out. The IGV_2.9.4 program was used to visualize sequence reads. MAFs were obtained from GnomAD, 1000 Genomes Project, Exome Sequencing Project, TopMED, and SEQ-specific cohorts comprising approximately 15,000 exome sequences of individuals from Turkiye with varying disorders. A set of *in silico* prediction tools, including FATHMM, M-CAP, CADD, SIFT4G, DANN, Polyphen-2, and Mutation Taster were used to examine the possible impact of selected variants on protein function. Human Splicing Finder (HSF Pro v3.1, Genomnis SAS Company) was used to evaluate the impact of splice region variants. The Genomic Evolutionary Rate Profiling (GERP) score was employed to estimate the evolutionary constraint in a particular position. Sanger sequencing was used to validate the variations obtained from the WES data and to perform segregation analysis. CLC Main Workbench 8.5 was used in Sanger sequence analysis against the reference sequence of the Ensembl GRCh37.p13 version. The list of primers used in Sanger sequencing are listed in Supplementary Table 2.

Results

Clinical Evaluations

Herein, we describe a family comprising a child with excess weight gain, his parents, and his grandmother (Figure 1). Currently, the BMIs of HP030 and HP031 were calculated

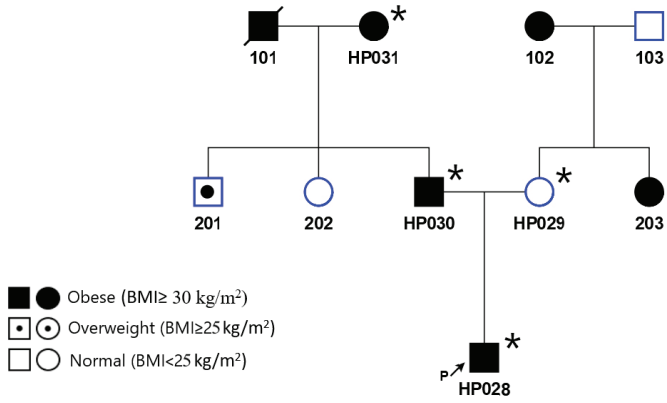

Figure 1. Detailed pedigree of the family with an obese infant. A three-generation family having an exclusively breastfed obese infant was analyzed in terms of obesity. The obese index case (7-month-old, male) was shown by an arrow. Asterisk indicates members with available genetic material. Body weight status was determined by WHO standards

WHO: World Health Organization

Table 1. Details of data quality and filtering results revealed by WES analysis

Quality metrics of whole exome sequencing data	
Total number of reads aligned	69.6M
(M = million)	
Average depth (%)	150.22
% Targets with 50X coverage	99.96
Total number of annotations	235.8K
(K = thousand)	
Total number of variants	35,554
Variants in candidate obesity genes	1,536
(Supplementary Table 1)	
Number of pathogenic variants*	10
Number of likely pathogenic variants*	6
Number of variants of uncertain significance (VUS)*	3,454
Homozygous variants	1,253
Heterozygous variants	23,024
Variant filtering for MAF ≤0.01	2,626

*Pathogenicity determined in accordance with ACMG guidelines.
WES was performed for the index case and exonic DNA was captured with the Twist Comprehensive Exome Kit (Twist Bioscience, South SF, CA, USA). Sequencing was performed on the Illumina Nextseq 550 platform with at least 20X reading depth. Numbers of obtained variants after filtering were shown in the table in different classifications.
WES: whole exome sequencing, VUS: variant of uncertain significance, ACMG: The American College of Medical Genetics and Genomics

Table 2. Details of detected variants in the index case by WES analysis

Gene	Variation	Amino acid change	dbSNP ID	Impact	MAF	Pathogenicity ACMG/SEQ	ClinVar	Mutation taster	SIFT	PolyPhen2	CADD score*	GERP score**
SH2B1	NM_001308293.1:c.28G>A	p.(Gly10Arg)	rs775528324	Missense	0.0000	VUS/VUS+	Not reported	Disease causing	Deleterious	Possibly damaging	23.3	4.46
PDE11A	NM_016953.4:c.919C>T	p.(Arg307Ter)	rs76308115	Stop gained	0.004	VUS/LP	Reported	Disease causing	NA	NA	36	5.47
CAPN10	NM_023083.4:c.84C>A	p.(Cys28Ter)	-	Stop gained	0.0000	LP/LP	Not reported	Disease causing	NA	NA	34	3.44
ADCY3	NM_001320613.2:c.1532C>T	p.(Ser511Leu)	rs139407103	Missense/ splice region	0.0005	LP/VUS	Not reported	Polymorphism	Tolerated	Tolerated	19.6	4.77

*Variants with a score CADD >20 are predicted to be among the 1.0% most deleterious possible substitutions in the human genome.
**GERP score is a measure of sequence conservation across multiple species. A score greater than 2 can be considered as evolutionary constrained.
dbSNP (www.ncbi.nlm.nih.gov/snp/), PolyPhen 2 (www.genetics.bwh.harvard.edu/pph2/).
MAF: minor allele frequency, VUS: variant of uncertain significance, LP: likely pathogenic, NA: not applicable, WES: whole exome sequencing, ACMG: The American College of Medical Genetics and Genomics

as 38.1 kg/m^2 (obese class II) and 31.3 kg/m^2 (obese class I), respectively, and HP031 had comorbidities of type 2 diabetes mellitus (T2DM) and hyperlipidemia. Moreover, the grandfather (#101), who died at the age of 61 due to complications caused by T2DM, was reported to be overweight. In contrast, the index's mother (HP029) was lean, whereas her sibling (#203) and her mother (#102) were both reported to be obese, and #102 was previously diagnosed with hypertension and T2DM.

The index case, HP028, was delivered at the 42nd gestational week by C-section with no other complications. His weight was 4,060 g (1.35 SDS), and his height was 52 cm (0.7 SDS) at delivery. He gained almost 1.5 kg per month as scaled on periodic examinations. He was admitted to our clinic when he was 7 months old, when his height and weight were measured as 74.2 cm (1.64 SDS), and 13.6 kg (4.07 SDS), respectively, and that weight-for-height was 4.25 SDS. His neuro-motor development was normal, and he showed no distinct facial or body features, making the possibility of a genetic syndrome less likely. Biochemical assessments showed no abnormalities in metabolic, thyroid, adrenal, or pituitary hormones. Hepatic ultrasound reported 2 centimeters of growth of the liver at the age of four months, which was within normal limits. During the first year of life, his weight reached 17 kg (4.22 SDS). He is still under yearly follow-up.

Whole Exome Sequencing Results

Quality metrics and filtering results achieved by WES are displayed in Table 1. WES analysis revealed four heterozygous variants in genes previously associated with obesity, which are detailed in Table 2. All variants were evaluated as novel candidates for obesity, except for the one found in *PDE11A*, which was previously associated with obesity cases having high blood pressure (27). These candidate variants confirmed paternal inheritance in family segregation as shown in Figure 2.

Discussion

Obesity is a multifactorial disorder that is influenced by factors such as irregular energy balance, genetic predisposition, a sedentary lifestyle, and socioeconomic status (28). Obesity has become an epidemic and is increasingly observed, especially among pediatric cases, where genetic predisposition and environmental factors lead to obesity persisting into and through adulthood (6,29). Therefore, clinical follow-up to detect postnatal accelerated growth in the first two years of life, which is recognized as a critical period for the development of childhood obesity, may be important in the prevention of obesity and its disparities in adulthood (3,13,30).

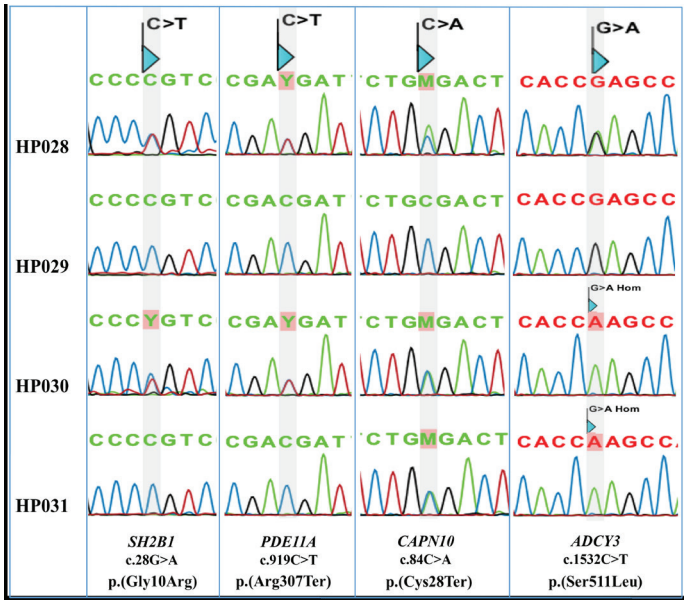

Figure 2. Confirmation of candidate variants by Sanger sequencing and family segregation analysis. Chromatograms of candidate genetic variants are shown in all available family members. Genetic changes are marked with triangles

In this study, we focused on the possible genetic cause of excess weight gain in an obese infant, who was exclusively breastfed. Paternal inheritance was suspected according to the family history, as his father and paternal grandparents were obese from childhood. Results obtained from WES analysis of the index identified four candidate genes that might be responsible for obesity in the members of this family. Among these candidates, two of them are stop-gained, while others are missense variations, as shown in Table 2. Paternal inheritance was confirmed for all these variants through family segregation.

The Src homology 2B adaptor protein 1 *(SH2B1)* p.(Gly10Arg) (rs775528324) novel missense variant was found in the heterozygous state in HP028 and HP030. This gene is involved in body weight regulation as a signaling molecule downstream of the leptin receptor. Disruptions in *SH2B1* were reported to cause anomalies in the digestive system and growth abnormalities, in addition to severe early-onset obesity-insulin resistance syndrome (2). Studies uncovered the causative effects of structural variants or SNPs in this gene as directly associated with increased BMI and severe early-onset obesity (17). According to *in vitro* functional experiments, *SH2B1* has multiple isoforms expressed in a variety of cells in the body. The *SH2B1β* isoform is mainly expressed in the hypothalamus, where body weight is regulated, and it also interacts with the leptin signaling pathway (31). *SH2B1* enhances insulin- and leptin-induced insulin receptor substrate 2 phosphorylation and growth hormone-induced cell motility (32). Despite the many causative variants in *SH2B1* linked to obesity to date, the Gly10Arg variant is described for the first time herein. We suggest that this variant is one of the candidates that might be involved in the obesity phenotype in this family, with high scores gathered from *in silico* predictions for evolutionary conservation by GERP (4.46), and for deleteriousness by CADD (23.30) combined.

In HP028 and HP030, the p.(Arg307Ter) rs76308115 stop-gained variant was detected in the phosphodiesterase 11A *(PDE11A)* gene, a member of the phosphodiesterase (PDE) family of genes. It is a null variant previously associated with obesity that causes loss of function (LOF) and the variant's pathogenicity is predicted to be very strong by *in silico* tools. Along with the Arg307Ter variant (MAF < 0.004), eight more pathogenic null variants in this gene were reported in ClinVar. A GERP score of 5.47 indicates a high conservation pattern for the Arg307 position. PDEs mediate cyclic-AMP (cAMP) degradation to AMP in the cAMP-dependent protein kinase (PKA) signaling pathway, which is involved in the regulation of energy balance through adipogenesis and lipogenesis (33,34). Thus, dysregulation of this pathway has been linked to obesity. Ohlsson et al. (27) previously described the role of *PDE11A* Arg307Ter variant in elevated blood pressure, high BMI, abdominal obesity, and the risk of ischemic stroke, in the Swedish population. Moreover, the Arg307Ter variant has been found in patients diagnosed with pigmented nodular adrenocortical disease and Cushing syndrome, in which obesity can be observed as a component (35,36). Nevertheless, functional validation of this variant is necessary to delineate its role in obesity pathogenicity.

The Adenylate cyclase 3 (*ADCY3)* p.(Ser511Leu) (rs139407103) variant is a novel missense splice-site variant. The impact of this change was reported by Human Splicing Finder (HSF Pro v3.1, Genomnis SAS Company), a splice site predictor tool as a significant alteration of exonic splicing enhancer/exonic splicing silencer motifs ratio. The variant was found to be heterozygous in HP028, while being homozygous in HP030 and HP031. This gene is known to be associated with obesity and BMI Quantitative Trait Locus 19 (BMIQ19), which consists of hyperlipidemia, hyperglyceridemia, and insulin resistance. Adenylate cyclase has a crucial role in the cAMP-dependent PKA signaling pathway by facilitating the production of cAMP from ATP (37). SNPs in the *ADCY3* gene are strongly associated with obesity (38) and it was shown that selective removal of *Adcy3* from the hypothalamus of a mouse led to evident body fat mass gain (39). Saeed et al. (40) suggested that recessive deleterious mutations in *ADCY3* caused monogenic severe obesity, using their data obtained from genetic and functional studies. It

was determined that deep RNA sequencing among homozygous and heterozygous carriers of an *ADCY3* splice-site variation caused severe and intermediate decrement in RNA expression levels, respectively (41). Variations causing splice site disruptions may initiate exon skipping or intron retention, which in turn might impair *ADCY3* function through generating different isoforms. Therefore, the impact of the novel Ser511Leu splice-site variant on both RNA expression and novel isoform generation merits further evaluation.

The Calpain-10 (*CAPN10)* p.(Cys28Ter) stop-gained novel variant was confirmed in HP028, HP030 and HP031. It is a null variant with LOF effects with extremely low frequency in the gnomAD population databases (MAF = 0.0000), which has not previously been reported in ClinVar. Diabetes mellitus, insulin-stimulated glucose uptake, dyslipidemia, adipose tissue disorders, and excess weight gain are known to be associated with *CAPN10*. Functional studies suggest that Calpain-10 is involved in the regulation of glucose homeostasis by participating in the remodeling of the cytoskeleton and catalyzing the translocation of GLUT4 (42). Polymorphisms in this gene were found to play roles in the thermogenesis and beta (3)-adrenoceptor function of obese individuals by reducing lipolytic sensitivity (43). The previously identified C-allele of SNP-44 in *CAPN10* was associated with elevated BMI and obesity, especially in the Chinese population and Turkish T2DM patients (44,45). While particular SNPs were found to regulate lipid metabolism, and lipogenesis in adipocytes, hence contributing to obesity (46), other SNPs were associated with lower BMI rates, particularly in Japanese populations (47). Prior knowledge of this gene concerning obesity along with predictions of LOF by *in silico* tools strongly suggests a role of this variant in the onset of obesity in this family. The results have confirmed the paternal inheritance of all potentially deleterious obesity-related variants. As the functional significance of most of these variants is not fully elucidated, it is presumed that the cumulative effect of these individual SNPs might explain the obesity phenotype observed in this family. Therefore, bearing in mind the father's (HP030) excess weight gain starting in childhood and the paternal inheritance of obesity-related genetic variants detected in this family, genetic counseling was provided to the index (HP028). In this respect, HP028 was predicted to be at increased risk for later obesity, so he should be under regular follow-up accordingly.

Prenatal and perinatal (fetal and early postnatal) influences, such as maternal age and eating habits, the existence of maternal metabolic disorders, intrauterine malnourishment, and even maternal smoking addictions were reported to interact markedly with infant adiposity. Thus, in the intrauterine period, the presence of gestational diabetes mellitus, and insufficient intrauterine nourishment were reported to be causative factors in obesity development (5,48,49). However, interviews with the mother (HP029) informed us of the absence of all these pre- and perinatal risk factors. Her BMI was in normal range (BMI: 22.7 kg/m^2) during and after pregnancy, and her weight gain during pregnancy was within acceptable limits. She did not manifest any metabolic disorders that appeared before, during, or after her labor, nor did she smoke. Hence, we believe that intrauterine risk factors can be excluded for this infant. In terms of postnatal influences, the infant was exclusively breastfed, the fact behind the rationale for this study focusing on delineating genetic components of obesity. However, interviews with the parents revealed that they followed a sedentary life, in which their high BMI and resistance to losing weight could probably have resulted from a combined effect of both lifestyle and the genetic variants they were found to carry.

Study Limitations

Our study is limited since it lacks functional validations, but nevertheless provides some novel, potentially obesity-related genetic variants. So, despite the limitation of small sample size due to inclusion of a single family, the power of detecting rare pathogenic variants, as a result of decreased gene pool, is important in identifying the genetic aspects of complex disorders. Therefore, by investigating a family at high-risk for obesity, we have been able to identify novel genetic variants that have the potential to be the causative variants in this family. However, these suggestions need to be validated, both functionally and by independent cohort studies, in order to assign definite roles to these variants in the pathogenesis of obesity.

Conclusion

Obesity is a complex disorder that involves both genetic and environmental risk factors. Herein, we described three generations of a family having an obese infant, who was exclusively breastfed, suggesting a genetic background for his phenotype and hence the rationale for the study. The clinical and genetic analyses revealed a paternal inheritance of obesity-related variants that may have influenced the condition in this baby. However, the family was advised about the need to exercise regularly in addition to adapting a healthy and balanced diet, due to the complex nature of obesity. Moreover, with the identified genetic risks factors, the infant will undergo regular follow-up assessments to prevent potential health issues. This proactive approach

aims to enable early interventions and personalized care strategies, thereby promoting the child's long-term well-being and development.

Ethics

Ethics Committee Approval: This study was approved by Istanbul University, Istanbul Medical Faculty Clinical Research Ethics Committee (protocol no: 2020/1054, date: 01.09.2020).

Informed Consent: Written and oral informed consents were taken from the family members or legal representatives.

Presented in: The findings of this study were presented as a poster at the European Society of Human Genetics Conference 2023.

Footnotes

Authorship Contributions

Surgical and Medical Practices: Ayşe Pınar Öztürk, Şükran Poyrazoğlu, Concept: Hazal Banu Olgun Çelebioğlu, Şükran Poyrazoğlu, Feyza Nur Tuncer, Design: Hazal Banu Olgun Çelebioğlu, Şükran Poyrazoğlu, Feyza Nur Tuncer, Data Collection or Processing: Hazal Banu Olgun Çelebioğlu, Ayşe Pınar Öztürk, Feyza Nur Tuncer, Analysis or Interpretation: Hazal Banu Olgun Çelebioğlu, Feyza Nur Tuncer, Literature Search: Hazal Banu Olgun Çelebioğlu, Writing: Hazal Banu Olgun Çelebioğlu, Şükran Poyrazoğlu, Feyza Nur Tuncer.

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