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Research Article

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

Olgun Celebioglu HB et al. Whole Exome Sequencing Revealed Genetic Variants in an Exclusively Breastfed Obese 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) was adopted in 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 utilized in predicting the risk for later obesity of an exclusively breastfed infant, and in providing genetic counseling to this family. The paternal inheritance of all potentially deleterious novel obesity-related variants was confirmed in the family.

Abstract
Objectives: Obesity is a serious health problem, that progressively affects individuals' lives with comorbidities involving heart disease, stroke, and diabetes mellitus. Since its prevalence increases particularly in children under age–of-five years, its genetic and environmental causes should be determined for prevention and control of the disease. This study aimed 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 indexcalculations were performed on available family members. Whole exome sequencing was performed on 7-month-oldobese infant utilizing Illumina-NextSeq550. Bioinformatic analyses were performed on the Genomize SEQ platform with variant filtering at minor allele frequencies (MAF)<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 (4.25SDS weight-for-height) was obvious in index case, where his father and grandmother were also obese (BMIs: 38.1kg/m^2 and 31.3kg/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 under periodic follow-up due to increased risk for later childhood obesity.
Keywords: Early-onset obesity, whole exome sequencing, paternal inheritance, novel variants, body mass index (BMI)

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Introduction

Obesity is a complex disorder characterized by excess body fat that manifests under the influence of 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 under the impact 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). Additionally, abnormalities linked to a single gene describe the rare monogenic/Mendelian form of obesity. Early-onset severe obesity (EOSO) is the development of obesity in early life, which might occur due to disruption in genes involved in pathways such as energy, 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 directly been related to the onset of adult obesity (6,8). Physiologic alterations in this critical development period contribute to the risk of obesity and comorbidities observed in adulthood (9). In this respect, infants born large for gestational age (LGA), along with rapid early growth and physical inactivity were determined as significant risk factors for obesity (10,11,12). Meta-analysis comprising twin, family, and adoption studies have stated 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 lead to severe pediatric obesity (3). Hence, genetic studies involving children are essential in determining 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

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(GWAS) have extensively been utilized to reveal common risk-conferring alleles, while whole exome sequencing (WES) was adopted in identifying novel rare variants in disease pathogenesis (17,18). Among these, GWAS exploits mostly non-coding single nucleotide polymorphisms (SNPs) to associate their impacts on the transcriptional regulation of nearby genes through comparing cases and controls. On the other hand, since 85% of mutations are determined to reside in protein-coding regions of the genome, WES has been utilized in detecting deleterious rare variants in disease pathogenesis (19). Therefore, GWAS and WES approaches complement each other in deciphering the missing heritability in complex disorders. In the case of obesity research, the collective efforts of these approaches explained only 2% to 6% of the genetic component of obesity in association with BMI variation (4,20), highlighting the need for further research in varying populations.

In this respect, we aimed to identify novel rare variants associated with early childhood obesity utilizing 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 utilized in predicting the risk for later obesity of this infant, and in providing genetic counseling to this family.

Materials and Methods

Patients and Clinical Assessments

A family from Turkiye, involving an exclusively breastfed 7-month-old 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 period of life. The grandmother reported a similar growth pattern about her son being obese starting in his early childhood and that it persisted 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 SDS according to WHO growth standards (21).

Weight, and height, and BMI standard deviation scores (SDS) of index case were calculated according to the growth chart prepared with national standards in the index case,and weight for height SDS according to WHO growth standards (22,23).BMI values of adult individuals were calculated by height, and weight respectively. Following WHO standards, BMI values greater than 25 were defined as obese and marked with black color on the pedigree (Figure 1). Written and oral 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). 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 Scientific Inc., MA, USA) 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, School 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 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 utilizing VEP v102 (26). All variant prioritizations were performed for 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 utilized in visualizing 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 utilized to examine the possible impact of selected variants on protein function. Human Splicing Finder (HSF Pro v3.1, Genomnis SAS Company) tool was utilized to evaluate the impacts 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 as 38.1kg/m^2 (Obese Class II) and 31.3kg/m^2 (Obese Class I), respectively, where 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 mentioned as overweight. On the other hand, the index's mother (HP029) was lean, whereas her sibling (#203) and her mother (#103) were both mentioned as obese, and #103 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 4060g (1.35 SDS), and his height was 52cm (0.7 SDS) at delivery. He gained almost 1.5kg per month as scaled on periodic examinations. He was admitted to our clinic when he was 7 months old, where 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, excluding the possibility of a genetic syndrome. Biochemical assessments showed no indication of abnormalities in metabolic, thyroid, adrenal, or pituitary hormones. Hepatic ultrasound found 2 centimeters of growth of the liver at the age of 4 months, which is within normal limits. During the first year of life, his weight reached up to 17 kg (6.27 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 candidates confirmed paternal inheritance in family segregation as shown in Figure 2.

Discussion

Obesity is a multifactorial disorder that is influenced by several factors such as irregular energy balance, genetic predisposition, a sedentary lifestyle, as well as socioeconomic aspects (28). Obesity has become an epidemic and is increasingly observed especially in pediatric cases, where genetic predisposition and environmental factors lead to adulthood obesity (6,29). Therefore, clinical follow-up to detect postnatal accelerated growth in the first 2 years of life, which is recognized as a critical period for the development of childhood obesity, could be important in the prevention of obesity and its disparities in adulthood (3,13,30).

In this study, we focused on the possible genetic cause of excess weight gain in an infant, who was exclusively breastfed. Paternal inheritance was suspected according to the family history, as his father and 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

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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 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. Especially *SH2B1β* isoforms are mainly expressed in the hypothalamus, where body weight is regulated, and they interact with leptin signaling pathway (31). *SH2B1* enhances insulin- and leptin-induced insulin receptor substrate 2 (IRS2) phosphorylation and GH-induced cell motility (32). Despite the plenty of causative variants in *SH2B1* linked to obesity so far, the Gly10Arg variant has been described for the first time herein. We suggest that this variant is one of the candidates that might be involved in 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.

On the other hand, in HP028 and HP030, p.(Arg307Ter) rs76308115 stop-gained variant was detected in phosphodiesterase 11A (*PDE11A)* gene, which is 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 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 Arg307 position. PDEs mediate the cAMP degradation to AMP in the Cyclic-AMP (cAMP)-dependent protein kinase (PKA) signaling pathway, which is involved in the regulation of energy balance through adipogenesis and lipogenesis (33,34). Thereby, dysregulations of this pathway were linked to obesity. In fact, Ohlsson *et al* previously depicted the role of *PDE11A* Arg307Ter variant in elevated blood pressure, high BMI, abdominal obesity, and the risk of ischemic stroke, in the Swedish population (30). 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 (ESE)/ exonic splicing silencer (ESS) 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 leads to obvious body fat mass gain (39). Saeed *et al.* advocated recessive deleterious mutations in *ADCY3* to cause monogenic severe obesity utilizing their data obtained from genetic and functional studies (40). 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 novel Ser511Leu splice-site variant's impact 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* gene. 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). Previously identified C-allele of SNP-44 in *CAPN10* gene 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 are 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. Obesity, as a complex disorder, cannot solely be explained by genetic risk factors. The influence of environmental risk factors on obesity is indispensable. 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 highly interact with infant adiposity. Accordingly, 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). In this respect, interviews with the mother (HP029) informed us of the absence of all mentioned risk factors above. Her BMI was in normal range (BMI: 22.7kg/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 she had smoking habits. Hence, intrauterine risk factors can be excluded for this infant. In terms of postnatal influences, the infant was exclusively breastfed, forming our rationale to focus on delineating genetic components of obesity. However, interviews with the parents revealed that they adapted a sedentary life, in which their high BMI and resistance to losing weight could probably resulted from a combined effect of both lifestyle and the genetic variants they were found to carry. Therefore, the family was informed about the need to exercise regularly in addition to adapt a healthy and balanced diet, while the infant needs to be followed-up regularly.

Moreover, further functional studies are required to improve our understanding of the potential role of novel variants determined in this family in the development of obesity. In this respect, our study is limited since it lacks functional validations but has a pivotal role in suggesting obesity-related novel genetic variants. In this respect, despite the limitation of small sample size due to the delineation of a single family, the power of detecting rare pathogenic variants, as a result of decreased gene pool, is invaluable in identifying the genetic aspects of complex disorders. Thereby, utilizing a high-risk family for obesity, we are suggesting novel genetic variants that have the potential of being 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 obesity pathogenesis.

Data Availability Statement

Data generated as part of this study are available from the corresponding author upon reasonable request.

Author Contribution Statement

All authors contributed equally to conducting this work, drafting, and revising the article, and have approved the final version of the manuscript. Among authors, HBOC took responsibility for WES analysis and confirmation of candidate variants, supervised by FNT. SP

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provided clinical evaluations of the patients, where APO helped with clinical follow-up, clinical data collection, and interviews with the parents. HBOC and FNT wrote the manuscript, while SP provided a critical review of the article. Funding was provided through FNT's project. The manuscript has not been and will not be submitted for publication elsewhere until the editorial board has decided whether to publish the article and the authors disclose no competing interests.

Ethical Approval

This study was approved by Istanbul University Medical Faculty Clinical Research Ethics Committee (Protocol no: 2020/1054); written and oral informed consents were taken from the family members or legal representatives.

Competing Interests

The authors declare no competing interests.

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References

1. Huvenne H, Dubern B, Clément K, Poitou C. Rare Genetic Forms of Obesity: Clinical Approach and Current Treatments in 2016. Obes Facts. 2016;9(3):158-173. doi:10.1159/000445061
2. Loos RJF, Yeo GSH. The genetics of obesity: from discovery to biology. Nat Rev Genet. 2022;23(2):120-133. doi:10.1038/s41576-021-00414-z
3. Littleton SH, Berkowitz RI, Grant SFA. Genetic Determinants of Childhood Obesity. Mol Diagn Ther. 2020;24(6):653-663. doi:10.1007/s40291-020-00496-1
4. SaeedS, Arslan M, Froguel P. Genetics of Obesity in Consanguineous Populations: Toward Precision Medicine and the Discovery of Novel Obesity Genes. Obesity (Silver Spring). 2018;26(3):474-484. doi:10.1002/oby.22064
5. Brisbois TD, Farmer AP, McCargar LJ. Early markers of adult obesity: a review. Obes Rev. 2012;13(4):347-367. doi:10.1111/j.1467-789X.2011.00965.x
6. Organization. WHO. Obesity and overweight. https://www.who.int/ news-room/fact- sheets/detail/obesity-and-overweight (accessed Sep. 10, 2020).
7. NCD Risk Factor Collaboration (NCD-RisC). Trends in adult body-mass index in 200 countries from 1975 to 2014: a pooled analysis of 1698 population-based measurement studies with 19.2 million participants. Lancet. 2016 Apr 2;387(10026):1377-1396.
8. Ong KK, Ahmed ML, Emmett PM, Preece MA, Dunger DB. Association between postnatal catch-up growth and obesity in childhood: prospective cohort study. BMJ. 2000 Apr 8;320(7240):967-71.
9. Dietz WH. "Adiposity rebound": reality or epiphenomenon? Lancet. 2000 Dec 16;356(9247):2027-8.
10. Woo Baidal JA, Locks LM, Cheng ER, Blake-Lamb TL, Perkins ME, Taveras EM. Risk Factors for Childhood Obesity in the First 1,000 Days: A Systematic Review. Am J Prev Med. 2016 Jun;50(6):761-779.
11. Moschonis G, Grammatikaki E, Manios Y. Perinatal predictors of overweight at infancy and preschool childhood: the GENESIS study. Int J Obes (Lond). 2008 Jan;32(1):39-47.
12. Lanigan J. Prevention of overweight and obesity in early life. Proc Nutr Soc. 2018 Aug;77(3):247-256
13. Stagi S, Bianconi M, Sammarco MA, Artuso R, Giglio S, de Martino M. New Thoughts on Pediatric Genetic Obesity: Pathogenesis, Clinical Characteristics and Treatment Approach. Adiposity Omics and Molecular Understanding. InTech; 2017.
14. Elks CE, den Hoed M, Zhao JH, Sharp SJ, Wareham NJ, Loos RJ, Ong KK. Variability in the heritability of body mass index: a systematic review and meta-regression. Front Endocrinol (Lausanne). 2012 Feb 28;3:29.
15. Bouchard C. Genetics of Obesity: What We Have Learned Over Decades of Research. Obesity (Silver Spring). 2021 May;29(5):802-820.
16. Paolacci S, Pompucci G, Paolini B, Del Ciondolo I, Miggiano GAD, Aquilanti B, Scaramuzza A, Velluti V, Matera G, Gagliardi L, Bertelli M. Mendelian non-syndromic obesity. Acta Biomed. 2019 Sep 30;90(10-S):87-89.
17. Yengo L, Sidorenko J, Kemper KE, Zheng Z, Wood AR, Weedon MN, Frayling TM, Hirschhorn J, Yang J, Visscher PM; GIANT Consortium. Meta-analysis of genome-wide association studies for height and body mass index in ~700000 individuals of European ancestry. Hum Mol Genet. 2018 Oct 15;27(20):3641-3649.
18. Akbari P, Lotta LA. et al. Sequencing of 640,000 exomes identifies GPR75 variants associated with protection from obesity. Science. 2021 Jul 2;373(6550):eabf8683
19. Kina BG, Topbas Selcuki NF, Bahat PY, et al. Whole exome sequencing reveals novel candidate variants for endometriosis utilizing multiple affected members in a single family. Mol Genet Genomic Med. 2024;12(1):e2312. doi:10.1002/mgg3.2312
20. Vourdoumpa A, Paltoglou G, Charmandari E. The Genetic Basis of Childhood Obesity: A Systematic Review. Nutrients. 2023 Mar 15;15(6):1416.
21. Grummer-Strawn LM, Reinold C, Krebs NF; Centers for Disease Control and Prevention (CDC). Use of World Health Organization and CDC growth charts for children aged 0-59 months in the United States [published correction appears in MMWR Recomm Rep. 2010 Sep 17;59(36):1184]. MMWR Recomm Rep. 2010;59(RR-9):1-15.
22. Neyzi O, Bundak R, Gökçay G, Günöz H, Furman A, Darendeliler F, Baş F. Reference Values for Weight, Height, Head Circumference, and Body Mass Index in Turkish Children. J Clin Res Pediatr Endocrinol. 2015 Dec;7(4):280-93.
23. Kurtoğlu S, Hatipoğlu N, Mazıcıoğlu MM, et al. Body weight, length and head circumference at birth in a cohort of Turkish newborns. J Clin Res Pediatr Endocrinol. 2012;4(3):132-139. doi:10.4274/jcrpe.693.
24. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics. 2009 Jul 15;25(14):1754-60..
25. Garrison E, Marth G. Haplotype-based variant detection from short-read sequencing. arXiv. 2016;1207.3907.
26. McLaren W, Gil L, Hunt SE, Riat HS, Ritchie GR, Thormann A, Flicek P, Cunningham F. The Ensembl Variant Effect Predictor. Genome Biol. 2016 Jun 6;17(1):122.
27. Ohlsson T, Lindgren A, Engström G, Jern C, Melander O. A stop-codon of the phosphodiesterase 11A gene is associated with elevated blood pressure and measures of obesity. J Hypertens. 2016 Mar;34(3):445-51.
28. Lin X, Li H. Obesity: Epidemiology, Pathophysiology, and Therapeutics. Front Endocrinol (Lausanne). 2021 Sep 6;12:706978.
29. Kelly AS. Current and future pharmacotherapies for obesity in children and adolescents. Nat Rev Endocrinol. 2023 Sep;19(9):534-541.
30. Díaz-Rodríguez M, Pérez-Muñoz C, Lendínez-de la Cruz JM, Fernández-Gutiérrez M, Bas-Sarmiento P, Ferriz-Mas BC. Effectiveness of a Multifactorial Intervention in the First 1000 Days of Life to Prevent Obesity and Overweight in Childhood: Study Protocol. Int J Environ Res Public Health. 2020 Mar 26;17(7):2239.
31. Ren D, Zhou Y, Morris D, Li M, Li Z, Rui L. Neuronal SH2B1 is essential for controlling energy and glucose homeostasis. J Clin Invest. 2007 Feb;117(2):397-406.
32. Giuranna J, Volckmar AL, Heinen A, Peters T, Schmidt B, Spieker A, Straub H, Grallert H, Müller TD, Antel J, Haußmann U, Klafki H, Rui L, Hebebrand J, Hinney A. The Effect of SH2B1 Variants on Expression of Leptin- and Insulin-Induced Pathways in Murine Hypothalamus. Obes Facts. 2018;11(2):93-108.

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33. London E, Bloyd M, Stratakis CA. PKA functions in metabolism and resistance to obesity: lessons from mouse and human studies. J Endocrinol. 2020 Sep;246(3):R51-R64.
34. Tong T, Shen Y, Lee HW, Yu R, Park T. Adenylyl cyclase 3 haploinsufficiency confers susceptibility to diet-induced obesity and insulin resistance in mice. Sci Rep. 2016 Sep 28;6:34179.
35. Horvath A, Boikos S, Giatzakis C, Robinson-White A, Groussin L, Griffin KJ, Stein E, Levine E, Delimpasi G, Hsiao HP, Keil M, Heyerdahl S, Matyakhina L, Libè R, Fratticci A, Kirschner LS, Cramer K, Gaillard RC, Bertagna X, Carney JA, Bertherat J, Bossis I, Stratakis CA. A genome-wide scan identifies mutations in the gene encoding phosphodiesterase 11A4 (PDE11A) in individuals with adrenocortical hyperplasia. Nat Genet. 2006 Jul;38(7):794-800.
36. Libé R, Horvath A, Vezzosi D, et al. Frequent phosphodiesterase 11A gene (PDE11A) defects in patients with Carney complex (CNC) caused by PRKAR1A mutations: PDE11A may contribute to adrenal and testicular tumors in CNC as a modifier of the phenotype. J Clin Endocrinol Metab. 2011;96(1):E208-E214. doi:10.1210/jc.2010-1704
37. Wu L, Shen C, Seed Ahmed M, Östenson CG, Gu HF. Adenylate cyclase 3: a new target for anti-obesity drug development. Obes Rev. 2016;17(9):907-914. doi:10.1111/obr.12430
38. Tian Y, Peng B, Fu X. New ADCY3 Variants Dance in Obesity Etiology. Trends Endocrinol Metab. 2018 Jun;29(6):361-363.
39. Cao H, Chen X, Yang Y, Storm DR. Disruption of type 3 adenylyl cyclase expression in the hypothalamus leads to obesity. Integr Obes Diabetes. 2016;2(2):225-228.
40. Saeed S, Bonnefond A, Tamanini F, Mirza MU, Manzoor J, Janjua QM, Din SM, Gaitan J, Milochau A, Durand E, Vaillant E, Haseeb A, De Graeve F, Rabearivelo I, Sand O, Queniat G, Boutry R, Schott DA, Ayesha H, Ali M, Khan WI, Butt TA, Rinne T, Stumpel C, Abderrahmani A, Lang J, Arslan M, Froguel P. Loss-of-function mutations in ADCY3 cause monogenic severe obesity. Nat Genet. 2018 Feb;50(2):175-179.
41. Grarup N, Moltke I, Andersen MK, et al. Loss-of-function variants in ADCY3 increase risk of obesity and type 2 diabetes. Nat Genet. 2018;50(2):172-174. doi:10.1038/s41588-017-0022-7
42. Turner MD, Cassell PG, Hitman GA. Calpain-10: from genome search to function. Diabetes Metab Res Rev. 2005 Nov-Dec;21(6):505-14.
43. Hoffstedt J, Näslund E, Arner P. Calpain-10 gene polymorphism is associated with reduced beta(3)-adrenoceptor function in human fat cells. J Clin Endocrinol Metab. 2002 Jul;87(7):3362-7.
44. Chen SF, Lu XF, Yan WL, Huang JF, Gu DF. Variations in the calpain-10 gene are associated with the risk of type 2 diabetes and hypertension in northern Han Chinese population. Chin Med J (Engl). 2007 Dec 20;120(24):2218-23.
45. Demirci H, Yurtcu E, Ergun MA, Yazici AC, Karasu C, Yetkin I. Calpain 10 SNP-44 gene polymorphism affects susceptibility to type 2 diabetes mellitus and diabetic-related conditions. Genet Test. 2008 Jun;12(2):305-9.
46. Pánico P, Salazar AM, Burns AL, Ostrosky-Wegman P. Role of calpain-10 in the development of diabetes mellitus and its complications. Arch Med Res. 2014 Feb;45(2):103-15.
47. Shima Y, Nakanishi K, Odawara M, Kobayashi T, Ohta H. Association of the SNP-19 genotype 22 in the calpain-10 gene with elevated body mass index and hemoglobin A1c levels in Japanese. Clin Chim Acta. 2003 Oct;336(1-2):89-96.
48. Larqué E, Labayen I, Flodmark CE, et al. From conception to infancy – early risk factors for childhood obesity. Nat Rev Endocrinol. 2019;15(8):456-478. Doi:10.1038/s41574-019-0219-1.
49. Oken E, Gillman MW. Fetal origins of obesity. Obes Res. 2003;11(4):496-506. doi:10.1038/oby.2003.69.

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.

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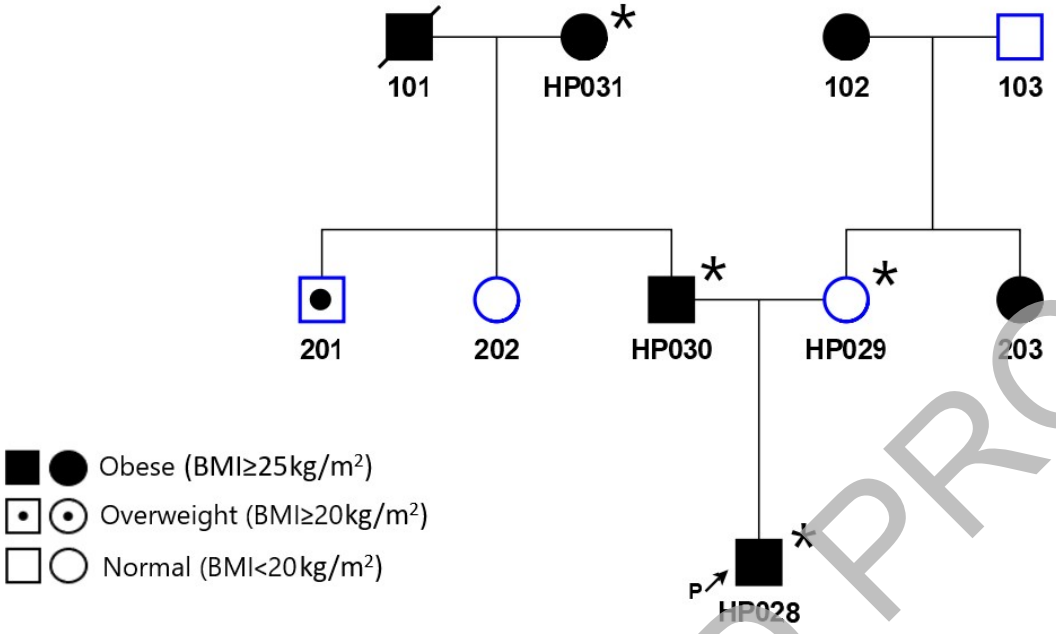
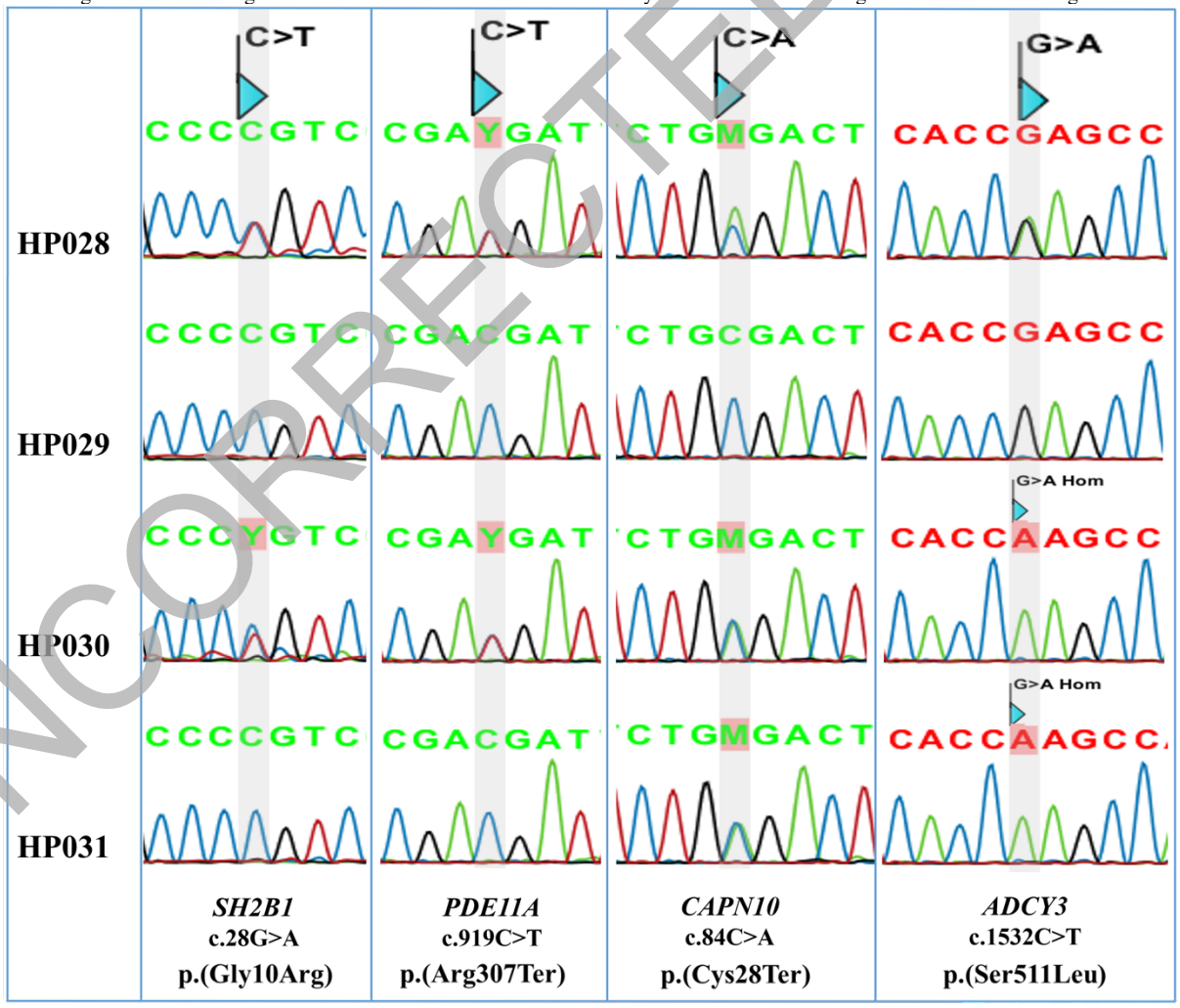


Figure 2: Confirmation of candidate variants by Sanger Sequencing and family segregation analysis
Chromatograms of candidate genetic variants are shown in all available family members. Genetic changes are marked with triangles.



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Tables:

Quality metrics of whole exome sequencing data

Total number of reads aligned *(M=million)*	69.6M
Average depth (%)	150.22
% Targets with 50X coverage	99.96
Total number of anotations *(K=thousand)*	235.8K
Total number of variants	35,554
Variants in candidate obesity genes *(Supplemental Table 1)*	1536
Number of pathogenic variants*	10
Number of likely pathogenic variants*	6
Number of variants of uncertain significance (VUS)*	3,454
Homozygous variants	12,53
Heterozygous variants	23,024
Variant filtering for MAF ≤ 0.01	2,626

**Pathogenicity determined in accordance with ACMG guidelines*

Table 1: Details of data quality and filtering results revealed by WES analysis
WES was performed on index case and exonic DNA was captured with the Twist Compherensive Exome Kit (Twist Bioscience South SF, 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.

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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	Poly-morphism	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/).

Abbreviations: MAF: Minor Allele Frequency, VUS: Variant Unknown Significant, LP: Likely Pathogenic, NA: Not Applicable

Supplementary Table 1: List of candidate genes for obesity

BDNF	Brain-derived neurotrophic factor
KSR2	Kinase suppressor of ras 2
ADCY3	Adenylyl cyclase 3
MC4R	Melanocortin 4 receptor
MC3R	Melanocortin 3 receptor
NTRK2	Neurotrophic tyrosine kinase receptor type 2
SH2B1	SH2B adaptor protein 1
SIM1	Single-minded (Drosophila) homologue 1
NR0B2	Nuclear receptor subfamily 0 group B member2
CEP19	Centrosomal protein 19 kDa
CPE	Carboxypeptidase E
HDAC8	Histone deacetylase 8 kDa
IGSF1	Immunoglobulin superfamily member1
LEP	Leptin

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LEPR	Leptin receptor
PCSK1	Proprotein conertase subtilisin/kexin type 1
POMC	Propiomelanocortin
TUB	Tubby bipartite transcription factor
MRAP2	Melanocortin 2 receptor accessory protein 2
ACP1	Acid phosphatase 1
DYRK1B	Dual specificity tyrosine phosphorylation regulated kinase 1B
PPARG	Peroxisome proliferative-activated receptor gamma
INSIG2	Insulin-induced gene2
INS	Insulin
ADIPOQ	Adiponectin
ADRA2A	Adrenergic receptor alfa-2A
ADRA2B	Adrenergic receptor alfa-2B
ADRB1	Adrenergic receptor ß-1
ADRB2	Adrenergic receptor ß-2
ADRB3	Adrenergic receptor ß-3
DRD2	Dopamine receptor D2
NR3C1	Nuclear receptor subfamily 3 group C member1
UCP1	Uncoupling protein 1
UCP2	Uncoupling protein 2
UCP3	Uncoupling protein 3
LIPE	Hormone sensitive lipase
CARTPT	Cocaine- and amphetamine-regulated transcript prepropeptide
ENPP1	Ectonucleotide pyrophosphatase/phosphodiesterase 1
PYY	Peptide tyrosine tyrosine
SDC3	Syndecan 3
NAMPT	Nicotinamide phosphoribosyltransferase
CFD	Complement factor D
SLC22A1	Solute carrier family 22 member 1
SLC2A4	Solute carrier family 2 member 4
SREBF1	Sterol regulatory element-binding transcription factor 1
PTPN1	Tyrosine-protein phosphatase non-receptor type 1
IRS-1	Insulin receptor substrate 1
GHRL	Ghrelin and obestatin prepropeptide
CCK	Cholecystokinin
NPY	Neuropeptide Y
NEGR1	Neuronal growth regulator 1
GIPR	Gastric inhibitory polypeptide receptor
TMEM18	Transmembrane protein 18 kDa
FTO	Fat mass- and obesity-associated gene
RETN	Resistin
ADD1	Alpha-adducin
AGRP	Agouti related neuropeptide

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Supp Table 1: The list of candidate genes known to be related to monogenic obesity was utilized for variant prioritization on bioinformatic analysis. These genes were found to have a direct association with obesity development in patients. Mutations in these genes, gene families, pathways, or interacted genes may contribute to obesity.

Supplementary Table 2: Primer list for Sanger Sequencing

(GRCh37/hg19) Assembly 5' > 3'	
Gene	Primer Sequence
SH2B1_F	GGAGTCTGAAGTAGGGTCGGA
SH2B1_R	CTCAAGGGACAGGTCATGTG
ADCY3_F	TCAGTCACCTCCATTGCAAC
ADCY3_R	TGAGATGGGAGTAAGTGCCATA
CAPN10_F	GACTCGCCTTCTCTCCG
CAPN10_R	CACGACCTAGTTTAGCGTCC
PDE11A_F	TGGAGCTCCTTCAGGAATCT
PDE11A_R	GGCAAACAACTATGGCATTG

Supp table 2: WES data analysis revealed final candidate gene variants in the index case, which were confirmed in the family members. Family segregation studies were also performed by utilizing these primer pairs in Sanger sequencing.

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