Genetic Causes of Severe Childhood Obesity: A Remarkably High Prevalence in an Inbred Population of Pakistan

Sadia Saeed,[1,2] Muhammad Arslan,[3] Jaida Manzoor,[4] Sadia M. Din,[5] Qasim M. Janjua,[5,6] Hina Ayesha,[7] Qura-tul Ain,[5] Laraib Inam,[3] Stephane Lobbens,[1] Emmanuel Vaillant,[1] Emmanuelle Durand,[1] Mehdi Derhourhi,[1] Souhila Amanzougarene,[1] Alaa Badreddine,[1] Lionel Berberian,[1] Stefan Gaget,[1] Waqas I. Khan,[8] Taeed A. Butt,[9] Amélie Bonnefond,[1,2] and Philippe Froguel[1,2]

Diabetes 2020;69:1424–1438 | https://doi.org/10.2337/db19-1238

Monogenic forms of obesity have been identified in ≤10% of severely obese European patients. However, the overall spectrum of deleterious variants (point mutations and structural variants) responsible for childhood severe obesity remains elusive. In this study, we genetically screened 225 severely obese children from consanguineous Pakistani families through a combination of techniques, including an in-house–developed augmented whole-exome sequencing method (CoDE-seq) that enables simultaneous detection of whole-exome copy number variations (CNVs) and point mutations in coding regions. We identified 110 (49%) probands carrying 55 different pathogenic point mutations and CNVs in 13 genes/loci responsible for nonsyndromic and syndromic monofactorial obesity. CoDE-seq also identified 28 rare or novel CNVs associated with intellectual disability in 22 additional obese subjects (10%). Additionally, we highlight variants in candidate genes for obesity warranting further investigation. Altogether, 59% of cases in the studied cohort are likely to have a discrete genetic cause, with 13% of these as a result of CNVs, demonstrating a remarkably higher prevalence of monofactorial obesity than hitherto reported and a plausible overlapping of obesity and intellectual disabilities in several cases. Finally, inbred populations with a high prevalence of obesity provide unique, genetically enriched material in the quest of new genes/variants influencing energy balance.

The monogenic forms of obesity have defined the current concepts of the central regulation of energy balance and have opened new avenues for precision medicine (1,2). Monogenic nonsyndromic obesity is due to pathogenic mutations in genes involved in leptin-melanocortin signaling, resulting in extreme, early-onset obesity with an insatiable craving for food (2). In addition to excessive adiposity, syndromic obesity associates with other abnormalities such as dysmorphic features, intellectual disability, and organ-specific anomalies (3).

Pathogenic variations causing severe obesity include not only point mutations but also copy number variations (CNVs) (4). We have recently developed a new strategy that is based on an augmented whole-exome sequencing (WES) method, named CoDE-seq, that enables accurate and cost-effective detection of point mutations and CNVs in one step, thus expediting comprehensive genetic diagnosis (5).

Although only 5–10% of severe, early-onset obesity cases in outbred populations have been evidenced to have a monogenic condition, we previously reported a high prevalence of homozygous mutations in *LEP*, *LEPR*, *MC4R*, and *ADCY3* in

[1]Université de Lille, INSERM UMR1283, CNRS-UMR 8199–European Genomic Institute for Diabetes, and Lille University Hospital, Lille, France
[2]Department of Metabolism, Digestion and Reproduction, Imperial College London, London, U.K.
[3]School of Life Sciences, Forman Christian College (A Chartered University), Lahore, Pakistan
[4]Department of Paediatric Endocrinology, Children's Hospital, Lahore, Pakistan
[5]Institute of Molecular Biology and Biotechnology, University of Lahore, Lahore, Pakistan
[6]Department of Physiology, University College of Medicine and Dentistry, University of Lahore, Lahore, Pakistan
[7]Department of Paediatrics, Punjab Medical College, Faisalabad, Pakistan
[8]The Children Hospital and the Institute of Child Health, Multan, Pakistan
[9]Department of Pediatrics, Fatima Memorial Hospital, Lahore, Pakistan

Corresponding author: Philippe Froguel, p.froguel@imperial.ac.uk, or Amélie Bonnefond, amelie.bonnefond@cnrs.fr

Received 16 December 2019 and accepted 25 April 2020

This article contains supplementary material online at https://doi.org/10.2337/figshare.12200843.

a Pakistani consanguineous population (6–9). Compared with other countries where consanguinity is practiced, the inbreeding coefficient reported for the Pakistani population is among the highest, with 60–65% of consanguineous marriages (10,11). Since Pakistan's population comprises several endogamous subethnic groups that are more or less isolated, the country has a very high genetic diversity, which optimizes comprehensive genetic studies, particularly in obesity because Pakistan has the ninth highest prevalence of obesity worldwide (12).

Here, we describe 110 (49%) genetically elucidated cases in the world's largest cohort of consanguineous subjects with severe, early-onset obesity ($n = 225$). Another 10% ($n = 22$) of obese case subjects were found to carry potentially causative CNVs associated with intellectual disability. Altogether, 59% of cases in the studied cohort are likely to have a genetic cause, with 13% of these potentially as a result of CNVs.

RESEARCH DESIGN AND METHODS

Participants

The investigation is based on 225 unrelated probands 0.2–22 years of age with severe, early-onset obesity and their family members from consanguineous families. Subjects were recruited on a voluntary basis from hospital pediatric units located in the province of Punjab, Pakistan. Patient/parent written informed consent was obtained for each subject. The study was approved by the institutional ethical committees. The selection criteria included a BMI SD score (SDS) for age ≥3 (using WHO Anthro version 3.2.2 and AnthroPlus), early-onset obesity and hyperphagia, and nonobese parents (with BMI of ≤30 kg/m^2) of first- or second-degree relation. Family and medical history was obtained, and pedigrees spanning at least three generations were constructed. Anthropomorphic measurements were made, and a blood sample was obtained for subsequent genomic DNA extraction and hormone estimations.

Initial Screening of *LEP* and *MC4R*

DNA from all probands ($n = 225$) was screened for coding regions of *LEP* and *MC4R* by direct sequencing as previously described (7).

WES and CoDE-seq

The probands found negative for mutations in *LEP* and *MC4R* ($n = 167$) and their family members ($n = 177$) were screened using WES and DNA arrays or CoDE-seq. A first batch of 62 probands and 56 family members were screened by standard WES and DNA arrays (for CNV detection) as previously described by us (9). A second batch of 105 probands and 121 family members were screened with CoDE-seq (when the technology was available and validated). This technique combines standard capture targeting the whole exome (NimbleGen SeqCap EZ MedExome Target Enrichment) with an in-house–designed capture (NimbleGen SeqCap EZ Choice XL) (5). Sequencing was performed on an Illumina NovaSeq 6000 system. A mean sequencing depth of ~100× was achieved for each individual using 150-bp paired-end reads. Computer analyses for variants detection and annotation have previously been described (5).

Variant Prioritization

The analysis, at first stage, was focused on homozygous variants. We excluded homozygous variants in obese probands that were also homozygous in family members with BMI ≤30 kg/m^2 or BMI SDS for age <2. The variants with an allele frequency of >0.001% in the Genome Aggregation Database (gnomAD) were also ignored. The resulting list of rare mutations was searched in a list of known monogenic obesity genes (Supplementary Table 1). Subsequently, rare compound heterozygous variants (in genes with recessive inheritance) and heterozygous variants (in genes with known dominant inheritance) were also analyzed. All potentially causative variants were graded according to criteria of the American College of Medical Genetics and Genomics (13).

CNV Detection and Prioritization

For detection of CNVs from the genotyping data, integrated hidden Markov model algorithm (PennCNV) with its default method was used as described previously (8,14). For detection of CNVs from CoDE-seq data, we used eXome Hidden Markov Model (XHMM, version 1.0) as previously described (5,15). We excluded all CNVs with allele frequency >0.002 in the gnomAD structural variant data set. Furthermore, we looked for all well-known pathogenic CNVs on the basis of the literature, the Database of Chromosomal Imbalance and Phenotype in Humans Using Ensembl Resources (DECIPHER), and the Database of Genomic Variants, as listed in our previous work (5).

Biochemical Analysis

Serum leptin, insulin, and cortisol concentrations were determined by commercially available ELISA kits. Assays were performed in duplicate with an automated analyzer (Bio-Rad Laboratories, Hercules, CA). The intra- and interassay variations were <11%.

Data and Resource Availability

The data sets generated during the current study are available from the corresponding author upon reasonable request. No applicable resources were generated or analyzed during the current study.

RESULTS

The genetic screening of 225 unrelated obese subjects, initially by Sanger sequencing (for *LEP* and *MC4R* only) followed by WES or CoDE-seq, revealed 55 pathogenic genetic variants (including point mutations or CNVs) in 110 probands (49% of the overall cohort) (Tables 1 and 2 and Fig. 1).

LEP: 47% of Elucidated Cases

We identified seven homozygous pathogenic mutations in *LEP* carried by 52 probands (Table 1 and Fig. 1). These included two frameshift (p.G133Vfs*15 and p.Y140Tfs*8), one in-frame (p.I35del), one splice site (c.-29+1G>C), and three missense variations (p.D100N, p.N103K, and p.R105Q). Of these mutations, c.-29+1G>C (identified in six unrelated probands) and p.Y140Tfs*8 (identified in one proband) were novel (i.e., not reported in the literature or listed in gnomAD) (Supplementary Fig. 1). The remaining five *LEP* mutations

Table 1—Genetic and clinical data of probands with a pathogenic point mutation in genes associated with obesity

Gene	Mutation (zygosity)	ID	Carriers, *n*	Patho-genicity	MAF in gnomAD	Sex	Age (years)	BMI SDS for age (children) BMI (adults)	Phenotype	Leptin (ng/mL)	Insulin (μIU/mL)	Cortisol (μg/dL)
LEP	c.-29+1G>C/p.? (hmz)	#132, #155, #237, #255, #297, #342	6	P	0	M: 2 F: 4	3.9 ± 0.9	9.1 ± 0.9	Excessive adiposity: #297, #342; hepatomegaly: #342; splenomegaly: #237; sleep apnea: #237, #342; delayed milestones	ND	9.2 ± 2.5	13.0 ± 1.0
LEP	c.398delG/ p.G133Vfs*15 (hmz)	#130, #134, #138, #143, #153, #157, #162, #172, #194, #195, #199, #206, #228, #241, #261, #264, #262, #276, #278, #279, #281, #284, #289, #292, #295, #296, #305, #311, #322, #323, #324, #325, #326, #330, #344, #352, #341, #127, #154	39	P	0.00003 (7/245,914)	M: 25 F: 14	2.0 ± 0.421 (#127)§ 25 (#154)§	8.4 ± 0.542 (#127) 50 (#154)	Excessive adiposity: #206; undescended testes: #228; hepatomegaly: #264, #295; dyspnea: #264; hypersomnia: #305, #341; recurrent RTI: #228, #261, #262, #279, #281, #289, #295, #296, #330, #352; delayed milestones	ND	21.8 ± 3.112 (#127) 36 (#154)	17.6 ± 1.331 (#127) 9 (#154)
LEP	c.104_106del/ p.I35del (hmz)	#345, #336	2	P	0.00001 (2/246,258)	M: 1 F: 1	10 (#345) 1.3 (#336)	3.7 (#345) 13.2 (#336)	Excessive adiposity: #345; polyuria	ND	26 (#345) 17 (#336)	12 (#345) 15 (#336)
LEP	c.309C>A/ p.N103K (hmz)	#300, #293	2	P	0.00175 (430/24,625)	F	1.5 (#300) 0.7 (#293)	7.2 (#300) 9.7 (#293)	Excessive adiposity, delayed milestones	32 (#300)Π 83 (#293)Π	17 (#300) 9 (#293)	14 (#300) 16 (#293)
LEP	c.314G>A/ p.R105Q (hmz)	#309	1	LP	0	F	1.8	6.7	Excessive adiposity, recurrent RTI	ND	5	9
LEP	c.298G>A/ p.D100N (hmz)	#249	1	P	0.00000 (1/246,244)	M	2.0	14.0	Excessive adiposity	12Π	11	12
LEP	c.417del/ p.Y140Tfs*8 (hmz)	#220	1	P	0	F	0.8	8.0	Excessive adiposity	ND	36	12
LEPR	c.2396–2A>G/p.? (hmz)	#183, #354	2	P	0	M: 1 F: 1	18 (#183)§ 0.7 (#354)	64 (#183) 1.3 (#354)	Excessive adiposity: #183; delayed milestones: #354; recurrent RTI	277 (#183) 136 (#354)	25 (#183) 19 (#354)	10 (#183) 15 (#354)

Continued on p. 1427

Table 1—Continued

Gene	Mutation (zygosity)	ID	Carriers, *n*	Patho-genicity	MAF in gnomAD	Sex	Age (years)	BMI SDS for age (children) BMI (adults)	Phenotype	Leptin (ng/mL)	Insulin (μIU/mL)	Cortisol (μg/dL)
LEPR	c.2396–1G>T/p.? (hmz)	#248, #321	2	P	0.00040 (1/250,546)	M: 1 F: 1	0.4 (#248) 0.6 (#321)	8.4 (#248) 7.5 (#321)	Excessive adiposity	38 (#248) 26 #(321)	13 (#248) 18 (#321)	15 (#248) 14 (#321)
LEPR	c.704–1G>A/p.? (hmz)	#331, #173	2	P	0	F	0.5 (#331) 2.8 (#173)	7.7 (#331) 11.4 (#173)	Excessive adiposity	31 (#331) 61 (#173)	23 (#331) 36 (#173)	15 (#331) 14 (#173)
LEPR	c.40G>A/p.E14K (hmz)	#14	1	P	0	M	2.3	10.6	Excessive adiposity	56	6	7
LEPR	c.2114G>A/ p.W705* (hmz)	#150	1	P	0	M	0.8	6.7	Excessive adiposity	30	12	5
LEPR	c.2899_2900insAT/ p.A967Dfs*7 (hmz)	#170	1	P	0	F	0.9	6.4	Excessive adiposity	10	8	16
LEPR	c.1738del/ p.E580Kfs *37(hmz)	#186	1	P	0	M	1.6	9.2	Excessive adiposity	19	13	16
LEPR	c.2T>C/p.? (hmz)	#OB-1	1	P	0	M	18.5§	38.6	Excessive adiposity	54	12	11
LEPR	c.2627C>T/ p.P876L (hmz)	#283	1	LP	0.00071 (2/282,696)	M	0.7	12.2	Excessive adiposity, recurrent RTI	31	1	23
LEPR	c.2153A>G/ p.N718S (hmz)	#142	1	LP†	0.00040 (1/251,374)	F	0.7	7.0	Excessive adiposity	45	19	8
LEPR	c.2213–3C>G/p.? (hmz)	#328	1	LP†	0	F	3.0	7.8	Excessive adiposity, anemia, delayed milestones, aggressive behavior	28	17	17
LEPR	c.3268_3269del/ p.S1090Wfs*6 (hmz)	#312	1	P	0	M	14	6.6	Excessive adiposity, diabetes, delayed milestones	31	15	18
MC4R	c.493C>T/ p.R165W (hmz)	#269, #273, #286, #310	4	P	0.00002 (6/246,054)	M: 1 F: 3	5.1 ± 1.7	6.7 ± 0.5	Excessive adiposity	22 ± 9	67 ± 22	16 ± 6

Continued on p. 1428

Table 1—Continued

Gene	Mutation (zygosity)	ID	Carriers, *n*	Patho-genicity	MAF in gnomAD	Sex	Age (years)	BMI SDS for age (children) BMI (adults)	Phenotype	Leptin (ng/mL)	Insulin (μIU/mL)	Cortisol (μg/dL)
MC4R	c.48G>A/p.W16* (hmz)	#202	1	P	0	M	5.3	13.2	Excessive adiposity	55	16	4
MC4R	c.47G>A/p.W16* (hmz)	#260, #337	2	P	0	M	4 (#260) 2 (#337)	7.4 (#260) 9.6 (#337)	Excessive adiposity	9 (#260) 11 (#337)	7 (#260) 26 (#337)	8 (#260) 13 (#337)
MC4R	c.482T>C/p.M161T (hmz)	#232	1	LP	0.000004 (1/246,124)	M	6.9	11.0	Excessive adiposity, hepatomegaly, hepatosteatosis, aggressive behavior	27	13	14
MC4R	c.633_636del/p.Y212Sfs*5 (hmz)	#233	1	P	0.000004 (1/246,020)	M	5.7	8.9	Excessive adiposity, bowlegs	10	8.6	9
MC4R	c.601_612del/p.F201_M204del (hmz)	#239	1	LP	0	F	7.2	4.8	Excessive adiposity, recurrent tonsillitis	14	13	11
MC4R	c.63_64del/p.Y21* (hmz)	#257	1	P	0.00001 (3/245,824)	F	4.6	8.0	Excessive adiposity	41	74	10
MC4R	c.206T>C/p.I69T (hmz)	#308	1	P	0.000004 (1/246,114)	F	2.5	6.4	Excessive adiposity	10	40	12
ADCY3	c.2173–10_2185del/p.? (hmz)	#306	1	P	0	F	1.2	6.7	Excessive adiposity	14	9	11
ADCY3	c.3315del/p.(I1106Sfs*3)‡ (hmz)	#107	1	P	0	F	15	3.5	Excessive adiposity, anosmia, amenorrhea, moderate intellectual disability	30	48	18
ADCY3	c.2578–1G>A/p.?‡ (hmz)	#158	1	P	0	M	6	6.5	Excessive adiposity	22	11	11
ADCY3	c.191A>T/p.N64I‡ (hmz)	#174	1	LP†	0.00026 (66/250,536)	M	6	6.5	Excessive adiposity, anosmia, moderate intellectual disability	11	18	7.5
BBS1	c.1570_1572del/p.N524del (htz); c.48–2A>C/p.? (htz)	#252	1	LP	0 and 0.00001 (3/246,272)	M	1.4	6	Excessive adiposity, polydactyl	9	16	15

Continued on p. 1429

Table 1—Continued

Gene	Mutation (zygosity)	ID	Carriers, *n*	Patho-genicity	MAF in gnomAD	Sex	Age (years)	BMI SDS for age (children) BMI (adults)	Phenotype	Leptin (ng/mL)	Insulin (μIU/mL)	Cortisol (μg/dL)
BBS1	c.432+1G>A/p.? (hmz)	#126	1	P	0.00001 (1/119,828)	F	3.2	6.5	Excessive adiposity, polydactyl	11	18	13
BBS1	c.1339G>A/ p.A447T (hmz)	#184	1	LP	0.00004 (10/250,660)	F	4	4	Excessive adiposity, polydactyl	10	22	18
BBS2	c.406dup/ p.A136Cfs*15 (hmz)	#93	1	P	0	M	9.2	4.5	Excessive adiposity	NA	47.5	NA
BBS2	c.116A>G/p.K39R (hmz)	#294	1	P	0.00001 (2/243,800)	M	11.0	5.0	Excessive adiposity, aggressive behavior, polydactyl	61	57	10
BBS2	c.1759_1762del/ p.P587Sfs*10 (hmz)	#318	1	P	0	M	9.0	6.2	Excessive adiposity, intellectual disability, polydactyl	27	19	10
BBS5	c.668_671del/ p.E223Afs*14 (hmz)	#316	1	P	0	F	10.3	5.6	Excessive adiposity, bowlegs, delayed milestones, poor vision, recurrent tonsillitis	14	86	11
BBS5	c.2T>A/p.? (hmz)	#198	1	P	0.000012 (3/249,784)	M	0.9	4.5	Excessive adiposity, polydactyl, recurrent RTI	16	11	1
BBS5	c.206T>G/p.V69G (hmz)	#335	1	LP†	0	M	14	4.1	Excessive adiposity, poor vision, intellectual disability, polydactyl	11	21	11
BBS9	c.662A>G/ p.E221G (htz), c.635T>C/ p.F212S (htz)	#128	1	LP	0	F	2.6	3.3	Excessive adiposity, polydactyl, recurrent RTI	14	25	9
BBS9	c.400del/ p.T134Qfs*5 (hmz)	#131	1	P	0	M	1.5	9.4	Excessive adiposity, polydactyl	15	17	NA
BBS10	c.271dup/ p.C91Lfs*5 (hmz)	#114, #73	2	P	0	M	1.1 (#114) 8.8 (#73)	4.9 (#114) 4.2 (#73)	Excessive adiposity, polydactyl	6 (#114) 42 (#73)	3 (#114) 4 (#73)	NA
BBS10	c.257T>C/ p.F86S(hmz)	#214	1	LP	0	M	0.6	4.3	Excessive adiposity	7	10	14

Continued on p. 1430

Table 1—Continued

Gene	Mutation (zygosity)	ID	Carriers, *n*	Patho-genicity	MAF in gnomAD	Sex	Age (years)	BMI SDS for age (children) BMI (adults)	Phenotype	Leptin (ng/mL)	Insulin (μIU/mL)	Cortisol (μg/dL)
MKKS	c.775del/ p.T259Lfs* 21(hmz)	#275, #258, #339	3	P	0.00004 (11/245,790)	F	1.3 (#258) 13 (#339)	10.8 (#258)2.6 (#339)	Delayed milestones; polydactyl: #258; sleep apnea: #339; poor vision in dark	25 ± 5	20 ± 5	12 ± 1
ALMS1	c.4937C>A/ p.S1646* (hmz)	#139	1	P	0.000004 (1/245,290)	M	2.5	6.2	Excessive adiposity	10	48	12
ALMS1	c.8008C>T/ p.R2670* (hmz)	#140	1	P	0	M	1.9	5.8	Excessive adiposity	8	39	17
ALMS1	c.7436C>G/ p.S2479* (hmz)	#221	1	P	0.00082 (2/245,262)	M	1.1	5.7	Anemia, poor vision	7	36	14
ALMS1	c.10975C>T/ p.R3659* (hmz)	#338	1	P	0.000004 (1/245,694)	M	2.0	8.6	Delayed milestones, poor vision	12	59	1

Data are mean ± SEM. hmz, homozygous; htz, heterozygous; LP, likely pathogenic; MAF, minor allele frequency; NA, not available; ND, not detectable (or <0.5 ng/mL); P, pathogenic; RTI, respiratory tract infection. §Young adults (≥18 years of age). ΠImmunoreactive but bioinactive leptin protein. †Upgraded from VUS to LP on the basis of strong phenotypic relevancy with regard to the genetic mutation. ‡Previously published by us (9).

Table 2—Genetic and clinical data of probands with CNVs causing obesity and/or intellectual disability

Proband ID	CNV	Genomic interval (hg38)	Gene(s)	Size (kb)	gnomAD region MAF Size	Patho-genicity	Gene or CNV-associated disorder	Age (years)	Sex	BMI SDS for age	Phenotype (other than obesity)	Additional pathogenic obesity-associated variants	Leptin (ng/mL)	Insulin (μIU/mL)	Cortisol (μg/dL)
#234	1q42.12 loss hmz	chr1:224923245–225051803	*DNAH14*	128.56	0	VUS	Panventriculomegaly (CNV del), intellectual disability, hydrops fetalis, nonimmune	2.4	F	9.4	Slow learner, intellectual disability		10	8	109
#238	1p31.3 loss hmz	chr1:65532214–65592881	*LEPR*	60.67	0	P	Morbid obesity	0.8	F	9.1			38	26	12
#263	1p31.3 loss hmz	chr1:65592634–65637041	*LEPR*	44.41	0	P	Morbid obesity	1.8	F	8.4	Delayed milestones, mental retardation		27	46	13
	7q31.1 loss htz	chr7:111337755–111407620	*IMMP2L*	69.87	chr7:111209525–111532640 0.00004656 323 kb	VUS	Autism, epilepsy, intellectual disability, multiple congenital abnormalities (CNV loss), neurodevelopmental disorder (CNV dup)								
#343	1q43 loss htz	chr1:240207914–240208747	*FMN2*	0.83	0	P	Intellectual disability, mental retardation, short stature, premature ovarian failure (CNV), intellectual disability	2.1	F	6.6	Delayed milestones		117	106	11
	7q31.1 loss htz	chr7:111180340–111569415	*IMMP2L*	389.08	0	P	Autism, epilepsy, intellectual disability, multiple congenital abnormalities (CNV loss), neurodevelopmental disorder (CNV dup)								
#304	10q21.3 loss htz	chr10:66402469–66676594	*CTNNA3*	274.13	0	VUS	Autism spectrum disorder (CNV), cardiomyopathy	3	M	7	NA		29	25	10
#334	10q25.3 loss htz	chr10:115799104–116272282	*ATRNL1. GFRA1*	473.18	0	VUS	*ATRNL1*: cognitive impairment, autism, dysmorphic features (CNV). *GFRA1*: Hirschsprung disease (CNV), central hypoventilation syndrome	2.7	F	5	Delayed milestones		22	19	5
#299	12p13.31 loss htz	chr12:9192171–9208382	*PZP*	16.21	chr12:9172788–9215201 0.00004656 42.4 kb	VUS	Autism spectrum disorder, congenital heart disease, neurodevelopmental disorder, breast cancer early onset	15	F	4	Metrorrhagia, severe body ache		40	23	12
#354	12q24.33 loss htz	chr12:133210742–133234403	*ZNF268. ANHX*	23.66	0	VUS	*ZNF268*: autism spectrum disorder, congenital heart disease	0.7	M	1.6	Frequent diarrhea	*LEPR* (c.2396–2A>G)	6	19	15
	7q22.2 loss htz	chr7:101039237–101041145	*MUC17*	1.91	chr7:101034793–101041958 0.0005203 7.17 kb	VUS	Autism spectrum disorder								
#271	14q11.2 loss htz	chr14:20350503–20357181	*PARP2*	6.68	0	VUS	Schizophrenia, prostate cancer, breast cancer	3.1	M	6	Slow learner		8	11	19
#339	16p13.3 loss htz	chr16:4959295–4992063	*SEC14L5*	32.77	chr16:4958364–5031681 0.00004656 73.3 kb	VUS	Autism spectrum disorder	13	F	5.5	Intellectual disability, delayed puberty, polydactyly	*MKKS* (c.775del. p.T259Lfs *21)	14	32	13
#347	16p12.2 loss htz	chr16:21510262–21728466	*OTOA. METTL9. IGSF6*	218.21	0	P	*OTOA*: autism spectrum disorder, hearing loss (CNV). *METTL9*: cognitive impairment	4	F	4.0	Delayed milestones, memory loss		4	25	14

Continued on p. 1432

Table 2—Continued

Proband ID	CNV	Genomic interval (hg38)	Gene(s)	Size (kb)	gnomAD region MAF Size	Patho-genicity	Gene or CNV-associated disorder	Age (years)	Sex	BMI SDS for age	Phenotype (other than obesity)	Additional pathogenic obesity-associated variants	Leptin (ng/mL)	Insulin (μIU/mL)	Cortisol (μg/dL)
#294	18p11.31 loss htz	chr18:4233040–4421599	*DLGAP1. DLGAP1-AS5*	188.56	0	VUS	*DLGAP1*: obsessive-compulsive disorder (CNV), schizophrenia, autism spectrum disorder, developmental disorder	11	M	5	Poor vision, aggressive behavior	*BBS2* (c.116A >G. p.K39R)	61	57	10
#303	19p13.2 loss htz	chr19:8904672–8906876	*MUC16*	2.21	chr19:8688936–9141019 0.00004656 453 kb	VUS	Autism spectrum disorder	0.4	M	5	NA		5	21	10
#247	19p13.2 loss htz	chr19:12398272–12431993	*ZNF443. ZNF799*	33.72	chr19:12386649–12433971 0.0002328 47.3 kb	VUS	*ZNF799*: autism spectrum disorder, Tourette syndrome	6.5	M	4.5	NA		10	10	17
#244	2q11.2 loss htz	chr2:97077521–97244014	*FAHD2B. ANKRD36*	166.49	0	VUS	*ANKRD36*: autism spectrum disorder, psychosis	22	M		PWS-like features, no facial hair		31	16	16
#270	2q32.1 loss htz	chr2:183023784–184276709	*NCKAP1. DUSP19. NUP35*	1,252.93	0	VUS	*NCKAP1*: autism spectrum disorder	4.1	M	8.4	Sleep apnea, aggressive behavior, recurrent RTI		22	61	7
#215	2q37.3 loss htz	chr2:239281474–239282694	*HDAC4*	1.22	chr2:239281331–239282793 0.001498 1.46 kb	VUS	Brachydactyly, mental retardation	1.1	M	6.5	Sleep apnea, gastric problems		23	83	14
#219	20p12.1 loss htz	chr20:14867224–15009042	*MACROD2*	141.82	chr20:14677820–15136935 0.00004656 459 kb	VUS	Early-onset obesity (CNV dup); Kabuki syndrome, ADHD, schizophrenia (CNV loss)	0.8	M	8.7	Slow learner		18	18	7
	17q24.2 loss htz	chr17:68145129–68515603	*ARSG. AMZ2. PRKAR1A. WIPI1. SLC16A6*	370.48	0	VUS	*ARSG*: Usher syndrome, neurodevelopmental disorder. *PRKAR1A*: Carney complex (CNV)								
#224	22q12.3 loss htz	chr22:32247382–32255353	*SLC5A4*	7.97	0	VUS	ADHD	9.5	M	4.1	Asthma, mild intellectual disability		12	12	6
	7q31.1 loss htz	chr7:111407385–111596185	*IMMP2L*	188.8	chr7:111380309–111597224 0.00004656 217 kb	P	Autism, epilepsy, intellectual disability, multiple congenital abnormalities (CNV loss), neurodevelopmental disorder (CNV dup)								
#277	6p22.3 loss htz	chr6:17754387–17781333	*KIF13A*	26.95	0	VUS	Autism spectrum disorder	0.6	F	7	Wakes up at night demanding food		28	9	28
	4q35.1-q35.2gain	chr4:186017165–186210660	*TLR3. FAM149A. CYP4V2*	193.5	0	VUS	*TLR3*: herpes simplex encephalitis/ encephalopathy; immunodeficiency. *FAM149A*: pulmonary arterial hypertension. *CYP4V2*: Bietti crystalline corneoretinal dystrophy (CNV loss); retinitis pigmentosa								

Continued on p. 1433

Table 2—Continued

Proband ID	CNV	Genomic interval (hg38)	Gene(s)	Size (kb)	gnomAD region MAF Size	Patho-genicity	Gene or CNV-associated disorder	Age (years)	Sex	BMI SDS for age	Phenotype (other than obesity)	Additional pathogenic obesity-associated variants	Leptin (ng/mL)	Insulin (μIU/mL)	Cortisol (μg/dL)
#298	6p22.3 loss htz	chr6: 17893182 –18021242	*KIF13A*	128.06	0	VUS	Autism spectrum disorder	11	F	3.8	Intellectual disability, hypothyroidism		25	31	11
	15q21.1 gain	chr15: 45106503 –45178335	*DUOX2. DUOXA1. DUOX1. SHF*	71.83	0	VUS	*DUOX2*: hypothyroidism. *DUOXA1*: schizophrenia, hydrops fetalis. *DUOX1*: intellectual disability, hypothyroidism								
#250	8p22 loss htz	chr8:16098745–16164282	*MSR1*	65.54	chr8:16087944–16166408 0.001723 78.5 kb	VUS	Schizophrenia (CNV loss), Barrett esophagus/esophageal adenocarcinoma, prostate cancer	0.9	F	3.7	Mild intellectual disability, slow movements		7	2	10
#316	10q26.13 gain	chr10:121503759–121870059	*FGFR2. ATE1*	366.3	0	VUS	*FGFR2*: Apert syndrome (CNV dup), craniosynostosis. *ATE1*: hearing impairment, tinnitus, atrioventricular septum defect	10.3	F	5.5	Slow learner, bowlegs, poor vision	*BBS5* (c.668_671 del. p.E223Afs *14)	14	86	11
#333	4p16.3 loss htz	chr4:3444014–3448310	*HGFAC*	4.3	0	VUS	Autism spectrum disorder	19	M	BMI: 39	Delayed milestones		45	102	14
#39	15q11.2-q13.1 loss htz	chr15:23235221–26108349 (hg19)	*MKRN3. MAGEL2. NDN. NPAP1. SNRPN. SNURF. UBE3A. ATP10A*	2,873.1	0	P	15q11.2-q13.1 loss CNV, feeding difficulties in infancy, hypogonadism, intellectual disability, muscular hypotonia, truncal obesity, schizophrenia	6.1	F	5.2	Atrophied uterus and ovaries		130	36	8
#87	15q11.2-q13.1 loss htz	chr15:23996462–28544359 (hg19)	*NPAP1. SNRPN. SNURF. UBE3A. ATP10A. GABRB3. GABRA5. GABRG3. OCA2. HERC2*	4,547.9	0	P	15q11.2-q13.1 loss CNV, feeding difficulties in infancy, hypogonadism, intellectual disability, muscular hypotonia, truncal obesity, schizophrenia	22	F	BMI: 43	No menarche, moderate intellectual disability		28	19	7
#320	15q11.2-q13.1 loss htz	chr15:22781870–26561186	*NIPA1. NIPA2. CYFIP1. TUBGCP5. GOLGA6L1. LOC102723534. GOLGA8S. MKRN3. MAGEL2. NDN. LOC105370733. NPAP1. SNRPN. SNURF. UBE3A. ATP10A. GABRB3*	3,779.320	0	P	15q11.2-q13.1 loss CNV, feeding difficulties in infancy, hypogonadism, intellectual disability, muscular hypotonia, truncal obesity, schizophrenia	4.8	M	6.0	PWS-like features, intellectual disability		12	28	7
	5q23.2 gain	chr5:127447531–127539157	*MEGF10. PRRC1*	91.63	chr5:127045431–127965806 0.00004902 920 kb	VUS	*MEGF10*: minicore myopathy, congenital myopathy, muscle weakness								

Continued on p. 1434

Table 2—Continued

Proband ID	CNV	Genomic interval (hg38)	Gene(s)	Size (kb)	gnomAD region MAF Size	Patho-genicity	Gene or CNV-associated disorder	Age (years)	Sex	BMI SDS for age	Phenotype (other than obesity)	Additional pathogenic obesity-associated variants	Leptin (ng/mL)	Insulin (μIU/mL)	Cortisol (μg/dL)
#282	16p11.2 loss htz	chr16:29581926–30231884	*SPN. QPRT. C16orf54. ZG16. KIF22. MAZ. PRRT2. PAGR1. MVP. CDIPT. SEZ6L2. ASPHD1. KCTD13. TMEM219. TAOK2. HIRIP3. INO80E. DOC2A. C16orf92. FAM57B. ALDOA. PPP4C. TBX6. YPEL3. GDPD3. MAPK3. CORO1A. BOLA2B. SLX1A. SULT1A3. NPIPB13*	649.96	0	P	16p11.2 loss CNV, severe obesity, autism	8	F	3.2	Slow learner, delayed milestones		18	7	16
#338	11p12 gain	chr11:36592557–36957106	*RAG2. C11orf74*	364.55	0	VUS	*RAG2*: immunodeficiency (CNV), Omenn syndrome	2	M	8.6	Poor vision, delayed milestones	*ALMS1* (c.10972C>T. p.R3658*)	12	60	1
#315	11q23.3 gain	chr11:118077018–118152698	*TMPRSS4. SCN4B*	75.68	chr11:118076058–118161781 0.00004902 85.7 kb	VUS	*TMPRSS4*: cerebral atrophy, autosomal recessive. *SCN4B*: atrial fibrillation	2.6	F	7.2	NA		15	25	10
#313	16p13.3 gain	chr16:43545–176835	*POLR3K. SNRNP25. MPG*	133.29	0	VUS	*POLR3K*: Hypomyelinating leukodystrophy. *MPG*: schizophrenia, colorectal cancer	5.2	F	5.5	Intellectual disability, recurrent RTI		11	50	13
#290	18q21.33 gain	chr18:63641736–63659552	*SERPINB3. SERPINB4*	17.82	0	VUS	*SERPINB3*: liver cirrhosis. *SERPINB4*: diabetes MODY, autism spectrum disorder	17	F		Secondary amenorrhea		31	96	11
#321	19q13.43 gain	chr19:57477251–57491801	*ZNF772. ZNF419*	14.55	chr19:57461161–57532262 0.00004902 71 kb	VUS	*ZNF419*: autism spectrum disorder	0.6	F	7.4	NA	*LEPR* (c.2396–1G>T)	26	17	13
#307	2p11.2 gain 4q35.1-q35.2 gain	chr2:85302449–85322813 chr4:186017165–186210660	*TCF7L1. TGOLN2* *TLR3. FAM149A. CYP4V2*	20.36 193.5	0 chr4:185990671–186121496 0.00009804 131 kb	VUS VUS	*TCF7L1*: glaucoma, primary congenital (CNV), autism *TLR3*: herpes simplex encephalitis/encephalopathy, influenza-associated. *CYP4V2*: Bietti crystalline corneoretinal dystrophy, retinitis pigmentosa. *TLR3*: inflammatory bowel disease, schizophrenia	6.0	M	5.2	Intellectual disability		17	34	11
#319	2q14.2 gain	chr2:119195166–119810001	*STEAP3. DBI. TMEM37. CFAP221. TMEM177. PTPN4*	614.84	0	VUS	*STEAP3*: hypochromic anemia. *PTPN4*: Rett-like syndrome (CNV loss), autism spectrum disorder, neurodevelopmental disorder	5	M	4.4	Delay in mental age, aggressive behavior		9	122	11
#285	21q11.2 gain	chr21:13251123–14109107	*POTED. LIPI*	857.99	0	VUS	*LIPI*: hypertriglyceridemia	11	M	3.7	NA		30	41	23
#275	21q22.3 gain	chr21:42259283–42296415	*ABCG1*	37.13	0	VUS	Abnormal HDL cholesterol, autism spectrum disorder, heart disease		F		NA	*MKKS* (c.775del. p.T259Lfs*21)	29	14	9

Continued on p. 1435

Table 2—Continued

Proband ID	CNV	Genomic interval (hg38)	Gene(s)	Size (kb)	gnomAD region MAF Size	Patho-genicity	Gene or CNV-associated disorder	Age (years)	Sex	BMI SDS for age	Phenotype (other than obesity)	Additional pathogenic obesity-associated variants	Leptin (ng/mL)	Insulin (μIU/mL)	Cortisol (μg/dL)
#342	22q11.22 gain	chr22:21955538–22110518	*TOP3B*	154.98	0	P	Mild mental retardation, generalized overgrowth (CNV dup), intellectual disability, schizophrenia	1.8	F	8.8	Delayed milestones	*LEP* (c.-29+1G>C)	2	19	15
	3q29 gain	chr3:197791254–197875785	*LRCH3*	84.53	chr3:197791715–197915689 0.00009804124 kb	VUS	Neurodevelopmental disorder								
#266	22q11.22 gain	chr22:21955538–22110518	*TOP3B*	154.98	0	P	Mild mental retardation, generalized overgrowth (CNV dup), intellectual disability, schizophrenia	4.4	F	6.1	NA		23	26	8
#329	22q11.22 gain	chr22:21957088–22215308	*TOP3B*	258.22	0	P	Mild mental retardation, generalized overgrowth (CNV dup), intellectual disability, schizophrenia	2.8	F	8.3	NA		19	26	8
#205	4p13 gain	chr4:42806238–43454858	*GRXCR1*	648.62	0	VUS	Deafness, dizziness	1.6	M	5.3	NA		5	9	5
#252	5q31.3 gain	chr5:141332929–141341211	*PCDHGA1. PCDHGA2*	8.28	0	VUS	*PCDHGA2*: Cardiovascular malformation		M						
#332	5q32 gain	chr5:149860860–149886410	*PDE6A*	25.55	chr5:149629797–150001191 0.00009804 371 kb	VUS	Retinitis pigmentosa, Leber congenital amaurosis	16	M	3.2	Rudimentary external genitalia, autistic		35	27	10

ADHD, attention deficit hyperactivity disorder; chr, chromosome; del, deletion; dup, duplicate; hmz, homozygous; htz, heterozygous; MAF, minor allele frequency; MODY, maturity-onset diabetes of the young; NA, not available; P, pathogenic; RTI, respiratory tract infection.

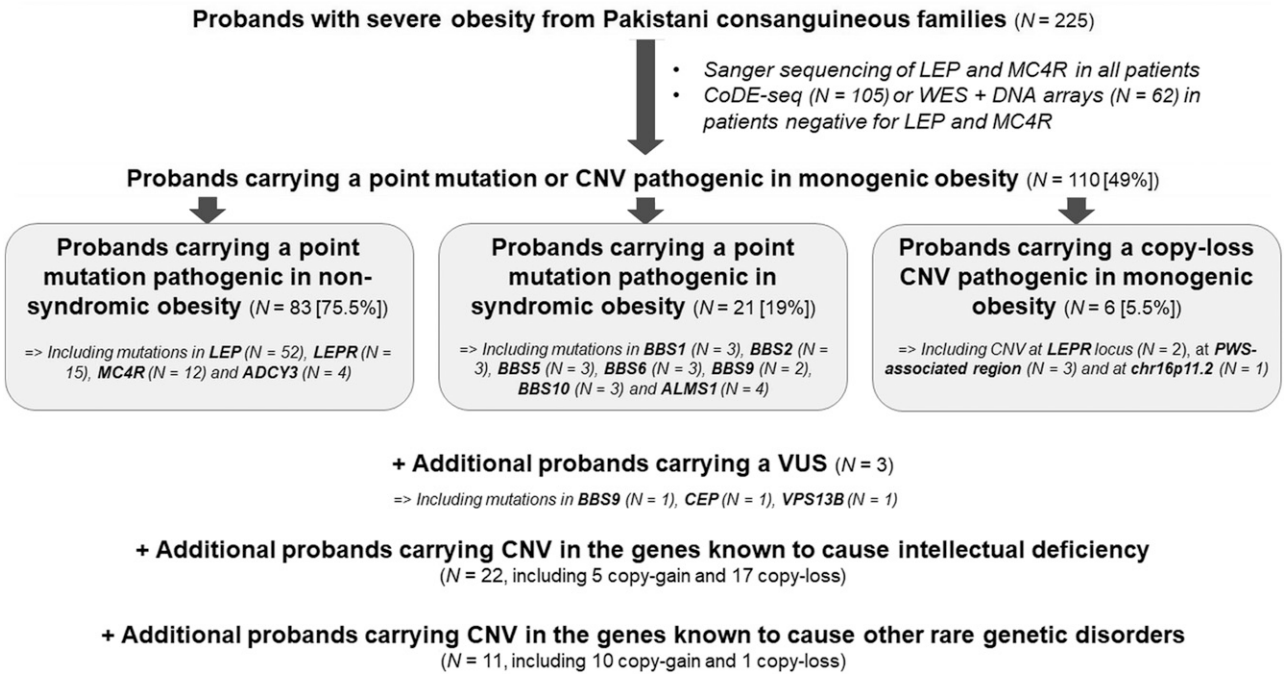

Figure 1—Genetic etiologies of obesity in a cohort of 225 patients from Pakistani consanguineous families.

have previously been reported (2). Of note, p.G133Vfs*15 was identified in 39 unrelated children and, by itself, accounted for obesity in 38% of the elucidated cases.

Besides excessive obesity and hyperphagia, the leptin-deficient probands often presented with respiratory infections, hepatomegaly, undescended testes, and delayed milestones. As anticipated, in leptin-deficient children, leptin levels were <0.2 ng or nondetectable (Supplementary Tables 2 and 3), with the exception of three probands with p.D100N and p.N103K variants with mean levels of 46 ± 12 ng/mL (Table 1 and Supplementary Table 4). Serum insulin and cortisol levels were variable but mostly in the higher range (Supplementary Tables 2–4).

LEPR: 15% of Elucidated Cases

We identified 14 homozygous pathogenic variants of *LEPR* in 17 probands (Tables 1 and 2 and Fig. 1). These included four splice-site (c.704–1G>A, c.2213–3C>G, c.2396–1G>T, and c.2396–2A>G), three frameshift (p.E580Kfs*37, p.A967Dfs*7, and p.S1090Wfs*6), one nonsense (p.W705*), and three missense mutations (p.E14K, p.N718S, and p.P876L); two copy-loss CNVs of 44.4 and 61 kb each; and a unique change at translation initiation site of *LEPR* (c.2T>C/p.?). Twelve of these *LEPR* variants were novel (Supplementary Fig. 2).

In contrast to previous findings (16), LEPR-deficient probands were phenotypically indistinguishable from leptin-deficient subjects. Apart from excessive obesity and hyperleptinemia (mean 41 ± 7.6 ng/mL, $n = 15$) (Supplementary Table 2), no other noticeable clinical problems except respiratory infection and/or delayed milestones in three subjects were reported.

MC4R: 11% of Elucidated Cases

We identified eight pathogenic homozygous mutations in *MC4R* in 12 probands that included three nonsense (c.47G>A/p.W16*, c.48G>A/p.W16*, and p.Y21*), one frameshift (p.Y212Sfs*5), one in-frame (p.F201_M204del), and three missense mutations (p.I69T, p.M161T, and p.R165W) (Table 1). Three of these variants were novel (Table 1 and Supplementary Fig. 3). In addition to excessive adiposity, hyperinsulinemia (44 ± 11 μIU/mL; $n = 12$) was recorded in most *MC4R*-deficient probands (Supplementary Table 2). No other significant clinical abnormalities were observed (Table 1).

Monogenic Syndromic Obesity (Bardet-Biedel Syndrome, *ALMS1*, and Prader-Willi Syndrome): 23% of Elucidated Cases

Twelve homozygous and two compound heterozygous mutations were identified in six genes linked to Bardet-Biedel syndrome (BBS) (*BBS1*, *BBS2*, *BBS5*, *MKKS* [*BBS6*], *BBS9*, and *BBS10*) in 17 probands (Table 1). The 12 homozygous mutations identified here included 6 frameshift, 2 splice-site, and 3 missense variants, and 1 was a substitution affecting the translation initiation codon (Table 1). Carriers of BBS gene mutations presented with central obesity and hyperphagia accompanied with other dysmorphic features (Table 1).

Four novel homozygous pathogenic, nonsense mutations in *ALMS1* were identified in four male subjects with severe obesity (Table 1). The majority of probands with these mutations presented hyperinsulinemia (Supplementary Table 2).

Furthermore, we identified three probands carrying heterozygous deletions in the 15q11–13 Prader-Willi syndrome

(PWS)–associated region (Table 2). Whereas one subject carried the typical 4.5-Mb deletion in the PWS-associated region, the other two subjects carried unique deletions of ~3.3 and ~3.8 Mb each (Supplementary Figs. 4–6). All three probands presented typical features of PWS (Table 1).

ADCY3: 4% of Cases

A girl with severe obesity was found with a unique 23-bp homozygous deletion (c.2173–10_2185del) in *ADCY3* (Table 1). In the same cohort, we have previously reported three other homozygous loss-of-function mutations in this gene (9).

Variants of Uncertain Significance: Point Mutations in Obesity Genes

We identified a homozygous novel mutation in *VPS13B* (p.P2207T), *CEP19* (p.M38T), and *BBS9* (p.Q748L). These mutations were variants of uncertain significance (VUS) (Supplementary Table 5).

Pathogenic Copy-Loss CNV in Chromosome 16p11.2

A proband was identified with an ~650-kb heterozygous deletion of chromosome 16p11.2 (Supplementary Fig. 7). Besides severe obesity, delayed milestones and intellectual disability were reported in the carrier.

CNVs Associated With Intellectual Disability and Potentially Causing Obesity (n = 22)

In addition to CNVs in known obesity-causing genes/regions, we found 47 CNVs, including 10 rare CNVs (allele frequency 0.001 in gnomAD) and 37 novel CNVs, in coding regions. These comprised 25 copy-loss and 11 copy-gain CNVs (Table 2). Of these, 36 CNVs have previously been associated with intellectual disability. Importantly, 28 of these 36 CNVs (21 copy loss and 7 copy gain) were found in 22 subjects who were negative for any other mutation (Table 2 and Fig. 1).

Novel CNVs Overlapping Genes Potentially Associated With Obesity (n = 4)

Four novel CNVs affecting candidate genes for energy balance impairment were also identified. This includes a small 8-kb heterozygous copy-loss CNV in *SLC5A4* (Table 2). *SLC5A4* is involved in the neuronal glucose sensing mechanism and control of food intake (17). Furthermore, we found a novel heterozygous 473-kb copy-loss CNV involving *ATRNL1*. This gene, mainly expressed in the brain, modulates the melanocortin signaling pathway (18). A 366.3-kb copy-gain CNV encompassing *FGFR2* and *ATE1* was identified in a proband also deficient for BBS5 (Table 1). *ATE1* is reported to affect adipogenesis and adipocyte function (19). Another 858-kb copy-gain CNV affecting the *LIPI* gene, which is involved in regulation of fat metabolism (20), was identified in a severely obese proband (Table 2).

DISCUSSION

The main result of this largest genetic study of patients with childhood-onset, severe obesity is genetic elucidation of obesity in 110 (49%) probands from a consanguineous population. This unexpectedly high percentage includes loss-of-function point mutations or CNVs in loci classically known to cause obesity. We confirm our earlier findings that leptin deficiency alone explains obesity in one-fifth of patients, making leptin deficiency the predominant etiology of monogenic obesity in this Pakistani population (7,8,21). The proportion of elucidated variants may even be much higher because we also identified an additional 22 cases with rare or novel CNVs linked to intellectual disabilities often associated with a severely obese phenotype. These cases provide credence to the notion that loci causing intellectual disability may also be involved in obesity (4). Thus, altogether, we elucidate up to 132 (59%) obesity cases, excluding severely obese patients in which VUS were identified in obesity genes (Supplementary Table 5).

This investigation highlights the importance of CNVs in the diagnosis of obesity. Although suggested by some of the pioneering studies (4,22), this hypothesis has so far received little attention from the clinical viewpoint (23) possibly because of the high cost of microarray technology, thus restricting its use to syndromic phenotypes only. In the current study, CoDE-seq has allowed us to directly detect CNVs in addition to point mutations (5). This comprehensive genetic screening in obesity is powerful because our retrospective clinical investigations unraveled the yet-undiagnosed intellectual disability in the probands carrying CNVs.

In conclusion, we show that next-generation sequencing approaches make it possible to uncover the genetic causality of severe obesity in a large proportion of subjects from the consanguineous population of Pakistan. An unrelenting quest for the discovery of new genes and variants, and associated pathways predisposing to obesity, is crucial in the development of specific and effective pharmacotherapies for the treatment of obesity.

Acknowledgments. The authors thank the patients and their families for participation in the study. The authors are grateful to H. Crouch (Imperial College London) for help in single nucleotide polymorphism array experiments and I. Qureshi (University of Lahore) for technical assistance. The authors also thank F. Allegaert and N. Larcher (CNRS-UMR 8199–European Genomic Institute for Diabetes) for DNA extraction and storage.

Funding. This study was supported by the Fédération de Recherche 3508 LabEx European Genomics Institute for Diabetes (ANR-10LABX-46), the ANR EquipEx 2010 session (ANR-10-EQPX-07-01, "LIGAN-PM"), the European Community (FEDER), and the Region Hauts-de-France. The research leading to this study was also supported by funding from the Pakistan Academy of Sciences (PAS003 to M.A.) and the European Research Council (GEPIDIAB 294785 to P.F.).

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

Author Contributions. S.S., M.A., S.M.D., Q.M.J., Q.A., and L.I. collected samples and performed biochemical analysis. S.S., M.A., A.Bo., and P.F. designed the study and wrote the first draft of the manuscript. S.S., E.V., E.D., M.D., S.A., A.Ba., L.B., and A.Bo. performed whole-exome sequencing and analyzed the genetic data. J.M., H.A., W.I.K., and T.A.B. identified and recruited obese families. S.L. carried out microarray experiments. S.G. managed the database. All authors contributed to the final version of the manuscript. S.S. and P.F. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

References

1. Clément K, Biebermann H, Farooqi IS, et al. MC4R agonism promotes durable weight loss in patients with leptin receptor deficiency. Nat Med 2018;24:551–555
2. Saeed S, Arslan M, Froguel P. Genetics of obesity in consanguineous populations: toward precision medicine and the discovery of novel obesity genes. Obesity (Silver Spring) 2018;26:474–484
3. D'Angelo CS, Kohl I, Varela MC, et al. Obesity with associated developmental delay and/or learning disability in patients exhibiting additional features: report of novel pathogenic copy number variants. Am J Med Genet A 2013;161A:479–486
4. Walters RG, Jacquemont S, Valsesia A, et al. A new highly penetrant form of obesity due to deletions on chromosome 16p11.2. Nature 2010;463:671–675
5. Montagne L, Derhourhi M, Piton A, et al. CoDE-seq, an augmented whole-exome sequencing, enables the accurate detection of CNVs and mutations in Mendelian obesity and intellectual disability. Mol Metab 2018;13:1–9
6. Saeed S, Bonnefond A, Manzoor J, et al. Novel LEPR mutations in obese Pakistani children identified by PCR-based enrichment and next generation sequencing. Obesity (Silver Spring) 2014;22:1112–1117
7. Saeed S, Butt TA, Anwer M, Arslan M, Froguel P. High prevalence of leptin and melanocortin-4 receptor gene mutations in children with severe obesity from Pakistani consanguineous families. Mol Genet Metab 2012;106:121–126
8. Saeed S, Bonnefond A, Manzoor J, et al. Genetic variants in LEP, LEPR, and MC4R explain 30% of severe obesity in children from a consanguineous population. Obesity (Silver Spring) 2015;23:1687–1695
9. Saeed S, Bonnefond A, Tamanini F, et al. Loss-of-function mutations in ADCY3 cause monogenic severe obesity. Nat Genet 2018;50:175–179
10. Hussain R, Bittles AH. The prevalence and demographic characteristics of consanguineous marriages in Pakistan. J Biosoc Sci 1998;30:261–275
11. Woods CG, Cox J, Springell K, et al. Quantification of homozygosity in consanguineous individuals with autosomal recessive disease. Am J Hum Genet 2006;78:889–896
12. Pigeyre M, Saqlain M, Turcotte M, Raja GK, Meyre D. Obesity genetics: insights from the Pakistani population. Obes Rev 2018;19:364–380
13. Richards S, Aziz N, Bale S, et al.; ACMG Laboratory Quality Assurance Committee. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 2015;17:405–424
14. Wang K, Li M, Hadley D, et al. PennCNV: an integrated hidden Markov model designed for high-resolution copy number variation detection in whole-genome SNP genotyping data. Genome Res 2007;17:1665–1674
15. Fromer M, Moran JL, Chambert K, et al. Discovery and statistical genotyping of copy-number variation from whole-exome sequencing depth. Am J Hum Genet 2012;91:597–607
16. Farooqi IS, Wangensteen T, Collins S, et al. Clinical and molecular genetic spectrum of congenital deficiency of the leptin receptor. N Engl J Med 2007;356:237–247
17. Gilles M. [Nutrient sensing by the gastro-intestinal nervous system and control of energy homeostasis]. Biol Aujourdhui 2015;209:325–330 [in French]
18. Haqq AM, René P, Kishi T, et al. Characterization of a novel binding partner of the melanocortin-4 receptor: attractin-like protein. Biochem J 2003;376:595–605
19. Singh A, Borah AK, Deka K, et al. Arginylation regulates adipogenesis by regulating expression of PPARγ at transcript and protein level. Biochim Biophys Acta Mol Cell Biol Lipids 2019;1864:596–607
20. Wen XY, Hegele RA, Wang J, et al. Identification of a novel lipase gene mutated in lpd mice with hypertriglyceridemia and associated with dyslipidemia in humans. Hum Mol Genet 2003;12:1131–1143
21. Saeed S, Bech PR, Hafeez T, et al. Changes in levels of peripheral hormones controlling appetite are inconsistent with hyperphagia in leptin-deficient subjects. Endocrine 2014;45:401–408
22. Bochukova EG, Huang N, Keogh J, et al. Large, rare chromosomal deletions associated with severe early-onset obesity. Nature 2010;463:666–670
23. Valsesia A, Macé A, Jacquemont S, Beckmann JS, Kutalik Z. The growing importance of CNVs: new insights for detection and clinical interpretation. Front Genet 2013;4:92