**Supplemental Material**

**Genetic causes of severe childhood obesity: a remarkably high prevalence (≥49%) in an inbred population of Pakistan**

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**Supplementary Tables**

**Supplementary Table 1.** List of genes in which point mutations have been shown to cause monogenic obesity

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Gene | Name | Location | Mendelian disorder | NM transcript |
| *ALMS1* | ALMS1, centrosome and basal body associated protein | 2p13.1 | Obesity & syndromic features | NM\_015120.4 |
| *ADCY3* | Adenylate Cyclase 3 | 2p23.3 | Obesity & syndromic features | NM\_004036.4 |
| *BBS1* | Bardet-Biedl syndrome 1 | 11q13.2 | Obesity & syndromic features | NM\_024649.4 |
| *BBS10* | Bardet-Biedl syndrome 10 | 12q21.2 | Obesity & syndromic features | NM\_024685.3 |
| *BBS12* | Bardet-Biedl syndrome 12 | 4q27 | Obesity & syndromic features | NM\_152618.2 |
| *BBS2* | Bardet-Biedl syndrome 2 | 16q13 | Obesity & syndromic features | NM\_031885.3 |
| *BBS4* | Bardet-Biedl syndrome 4 | 15q24.1 | Obesity & syndromic features | NM\_033028.4 |
| *BBS5* | Bardet-Biedl syndrome 5 | 2q31.1 | Obesity & syndromic features | NM\_152384.2 |
| *BBS7* | Bardet-Biedl syndrome 7 | 4q27 | Obesity & syndromic features | NM\_176824.2 |
| *BBS9* | Bardet-Biedl syndrome 9 | 7p14.3 | Obesity & syndromic features | NM\_001033604.1 |
| *BDNF* | brain derived neurotrophic factor | 11p14.1 | Obesity & syndromic features | NM\_170735.5 |
| *CEP19* | centrosomal protein 19 | 3q29 | Obesity & syndromic features | NM\_032898.4 |
| *CEP290* | centrosomal protein 290 | 12q21.32 | Obesity & syndromic features | NM\_025114.3 |
| *LEP* | leptin | 7q32.1 | Obesity | NM\_000230.2 |
| *LEPR* | leptin receptor | 1p31.3 | Obesity | NM\_002303.5 |
| *MC4R* | melanocortin 4 receptor | 18q21.32 | Obesity | NM\_005912.2 |
| *MKKS* | McKusick-Kaufman syndrome | 20p12.2 | Obesity & syndromic features | NM\_018848.3 |
| *MKS1* | Meckel syndrome, type 1 | 17q22 | Obesity & syndromic features | NM\_017777.3 |
| *MRAP2* | melanocortin 2 receptor accessory protein 2 | 6q14.2 | Obesity | NM\_138409.2 |
| *NTRK2* | neurotrophic receptor tyrosine kinase 2 | 9q21.33 | Obesity & syndromic features | NM\_006180.4 |
| *PCSK1* | proprotein convertase subtilisin/kexin type 1 | 5q15 | Obesity | NM\_000439.4 |
| *POMC* | proopiomelanocortin | 2p23.3 | Obesity | NM\_001035256.1 |
| *TUB* | tubby bipartite transcription factor | 11p15.4 | Obesity & syndromic features | NM\_003320.4 |
| *VPS13B* | vacuolar protein sorting 13 homolog B | 8q22.2 | Intellectual disability & obesity | NM\_017890.4 |

**Supplementary Table 2.** Physical and endocrine characteristics of children carrying point mutations

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Gene | Number of carriers | Sex | Agea (year) | BMI SDS for age | Leptinb (ng/ml) | Insulin (µIU/ml) | Cortisol (µg/ml) |
| *LEP* | 47 | M:26; F:21 | 2±0.4 | 8±0.4 | <0.2 | 21±2.6 | 17±1.1 |
| *LEPR* | 15 | M:7; F:8 | 2±0.9 | 8±0.7 | 41±7.6 | 18±2.9 | 13±1.1 |
| *MC4R* | 12 | M:6; F:6 | 5±0.6 | 8±0.7 | 22±5.1 | 44±11 | 12±2.4 |
| *ADCY3* | 1 | F | 1 | 7 | 14 | 9 | 11 |
| *CEP19c* | 1 | F | 2 | 5 | 16 | 9 | 11 |
| *BBS* | 15 | M:10; F:5 | 6±1.4 | 5±0.6 | 22±3.9 | 25±5.6 | 11±0.9 |
| *ALMS1* | 4 | M | 2±0.4 | 6±0.9 | 11±1.6 | 44±5.4 | 12±1.2 |

***Reference values:*** Probands with unrevealed genetic variants associated with severe obesity from the same cohort (n=20): Age (y): 2±0.3 y; BMI SDS: 6.7±0.8; Leptin (ng/ml) 7±0.8; Insulin (µIU/ml) : 28±7.5; Cortisol: (µg/ml) 13 ±1.0.

Wild-type children from the same population (n=15): Age (y): 5±0.4 y; BMI SDS: 0.2±0.3; Leptin (ng/ml): 3±0.3; Insulin (µIU/ml): 9.9±1.6; Cortisol (µg/ml): 11±0.6.

***Abbreviations:******BMI SDS:***body mass index standard deviation score*;* ***F:***female; ***M*:** male.

**a**Also see Supplementary Table 3.

**b**Also see Supplementary Table 4.

**c** VUS according to ACMG criteria.

**Supplementary Table 3.** Physical and endocrine characteristics of 4 adults carrying *LEP* and *LEPR* mutation

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Gene | Code | Sex | Age (year) | BMI  | Leptin (ng/ml) | Insulin (µIU/ml) | Cortisol (µg/ml) |
| *LEP* | #127 #154 | M | 21, 25 | 42, 50 | ND | 12, 36 | 31, 9 |
| *LEPR* | #183 #OB1 | F:1, M:1 | 18, 19 | 64, 39 | 177, 54 | 25, 12 | 10, 11 |

***Abbreviations:******BMI:***body mass index;***F*:** female; ***M*:** male; ***ND*:** not detectable.

**Supplementary Table 4.** Physical and endocrine characteristics of 3 leptin deficient children with bioinactive but with immunoreactive circulating leptin levels

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Gene | Code | Sex | Age (year) | BMI SDS for age | Leptin (ng/ml) | Insulin (µIU/ml) | Cortisol (µg/ml) |
| *LEP* | #300 #293 #249 | F:2, M:1 | 1±0.4 | 10±2 | 42±21.1  | 10±4.7 | 14±1.2 |

***Abbreviations:******BMI SDS:***body mass index standard deviation score*;* ***F*:** female; ***M*:** male.

**Supplementary Table 5** Genetic and phenotypic characteristics of severely obese probands with homozygous point mutations identified as VUS genes, from a consanguineous population

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Gene | ID’s | Mutation | MAF (GnomAD) | Gender | Age (years) | BMI SDS for age | Other clinical features | Insulin µIU/ml | Cortisol µg/dl |
| *BBS9*  | **#190** | c.2243A>T p.Q748L | 0.00006 (14/25087) | M | 19.0 | - | Hypertensive since 6 months of age | 107 | 13 |
| *CEP19*  | **#116** | c.113T>C p.M38T | 0 | F | 2 | 5.0 | Excessive adiposity | 9 | 11 |
| *VPS13B* | **#246** | c.6619C>A p.P2207T | 0.00001 (2/246040) | M | 1 | 7.9 | Hypotonia; delayed milestones | 8.9 | 2.2 |

***Abbreviations:******BMI SDS:***body mass index standard deviation score***; F*:** female; ***GnomAD*:** Genome Aggregation Database; ***M*:** male; ***MAF*:** minor allele frequency.

**Supplementary Figures**

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**Supplementary Fig 1**. Schematic presentation of homozygous *LEP* gene mutations identified in this study spanning three exons of the gene. The novel mutations identified are indicated in bold letters.



**Supplementary Fig 2**. Schematic presentation of full-length *LEPR*, indicating the location of the point mutations identified in individuals with severe early-onset obesity. The novel mutations identified in this study are indicated in bold letters.

***NTD***, N-terminal domain; ***CRH1*** and ***CRH2***, cytokine receptor homology domains; ***IGD***, immunoglobulin-like domain; ***FNIII***, membrane-proximal ﬁbronectin typIII domains; ***LC***, low complexity region

aMutation in initiation codon, bSplice site mutation.



**Supplementary Fig 3**. Schematic presentation of full-length *MC4R* showing seven transmembrane domains with 3 intracellular loops (ICL) and 3 extracellular loops (ECL) indicating the location of pathogenic mutations identified in individuals with severe early-onset obesity. The novel mutations identified in this study are indicated in bold letters.



**Supplementary Fig 4****.** Log R Ratio and B Allele Frequency Plot of a 3.3 Mb deletion in 15q11.2-q12 PWS associated region identified in #39. Multiplex Ligation-dependent Probe Amplification (MLPA) of patient DNA using P343 probemix was performed to confirm deletion breakpoint (data not shown).



**Supplementary Fig 5.** Log R Ratio and B Allele Frequency plot for a 4.5 Mb copy-loss CNV in 15q11.2-q12 PWS associated region found in #87. Multiplex Ligation-dependent Probe Amplification (MLPA) of patient DNA using P343 probemix was performed to confirm deletion breakpoint (data not shown).



**Supplementary Fig 6**. Identification and validation of a ~3.8 Mb copy-loss CNV in PWS associated region found in #320 detected by CoDE-seq. Copy-loss is highlighted in red.



**Supplementary Fig 7**. Identification and validation of a copy-loss CNV in 16p11.2 obesity associated region found in #282 detected by CoDE-seq. Copy-loss is highlighted in red.