Supplemental Material

Rare variant analysis of obesity-associated genes in young adults with severe obesity from a consanguineous population of Pakistan

Running title: Consanguinity and obesity linked rare variants

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Supplementary Tables

Supplementary Table 1. Physical and biochemical characteristics of the SOPP–Young Adults cohort (*n* = 128) and age-matched controls (n=50)

Characteristics	**SOPP – Young adults**	**Control subjects**
Age (year)	18.4 ± 0.3[a]	17.7 ± 0.3
Sex (M/F)	78 / 50	38/12
Height (cm)	163.9 ± 1.1	160.2 ± 4.2
Weight (kg)	101.0 ± 1.7***	63.1 ± 4.7
BMI (kg/m²)	37.2 ± 0.3***	19.0 ± 0.4
Insulin (µIU/ml)	30.4 ± 2.8***	10.0 ± 1.1
Leptin (ng/ml)	36.4 ± 1.6***	2.2 ± 0.2
Cortisol (µg/dl)	20.0 ± 0.8***	11.3 ± 0.3
Family history of obesity (%)	44.3	0.0
Family history of diabetes (%)	30.3	4.0

[a] Mean±SEM
Significantly different from control values ***$P<0.001$ (Student t test)
F, female; M, male
(In the SOPP children cohort 42.7% and 53% were reported with family history of obesity and diabetes, respectively).

Supplementary Table 2. List of genes in which point mutations were 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
SH2B1	SH2B adaptor protein 1	16p11.2	Obesity & insulin resistance	NM_001308293.1
SIM1	SIM BHLH Transcription Factor 1	6q16.3	Obesity & Prader-Willi-Like	NM_005068.3
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 3: ACMG criteria for classifying pathogenic or likely pathogenic variants in the present study

Evidence of pathogenicity	***Category***	
Very strong	**PVS1**	Null variant (nonsense, frameshift, initiation codon…)
Strong	**PS1**	Same amino acid change as a previously established pathogenic variant regardless of nucleotide change, *i.e.* Val→Leu caused by either G>C or G>T in the same codon (according to ClinVar ["likely pathogenic" or "pathogenic"] or HGMD database ["disease causing"])
Strong	**PS3**	Well-established in vitro or in vivo functional studies supportive of a damaging effect on the gene or gene product (according to HGMD database [functional characterization])
Moderate	**PM1**	Located in a mutational hot spot and/or critical and well-established functional domain (e.g., active site of an enzyme) without benign variation (according to ClinVar or HGMD database)
Moderate	**PM2**	Extremely rare in the general population (i.e. with less than one carrier according to gnomAD database [v2.1.1] [please see the number of homozygotes for genes following a recessive inheritance like *PCSK1*])
Moderate	**PM5**	Missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before (according to ClinVar or HGMD database)
Supporting	**PP2**	Variant in a gene that has a low rate of benign missense variation and in which missense variants are a common mechanism of disease
Supporting	**PP3**	Multiple lines of computational evidence (using PolyPhen-2 (HumDiv), SIFT and Mutation Taster) support a deleterious effect of the missense variant
Supporting	**PP4**	Patient's phenotype or family history is highly specific for a disease with a single etiology

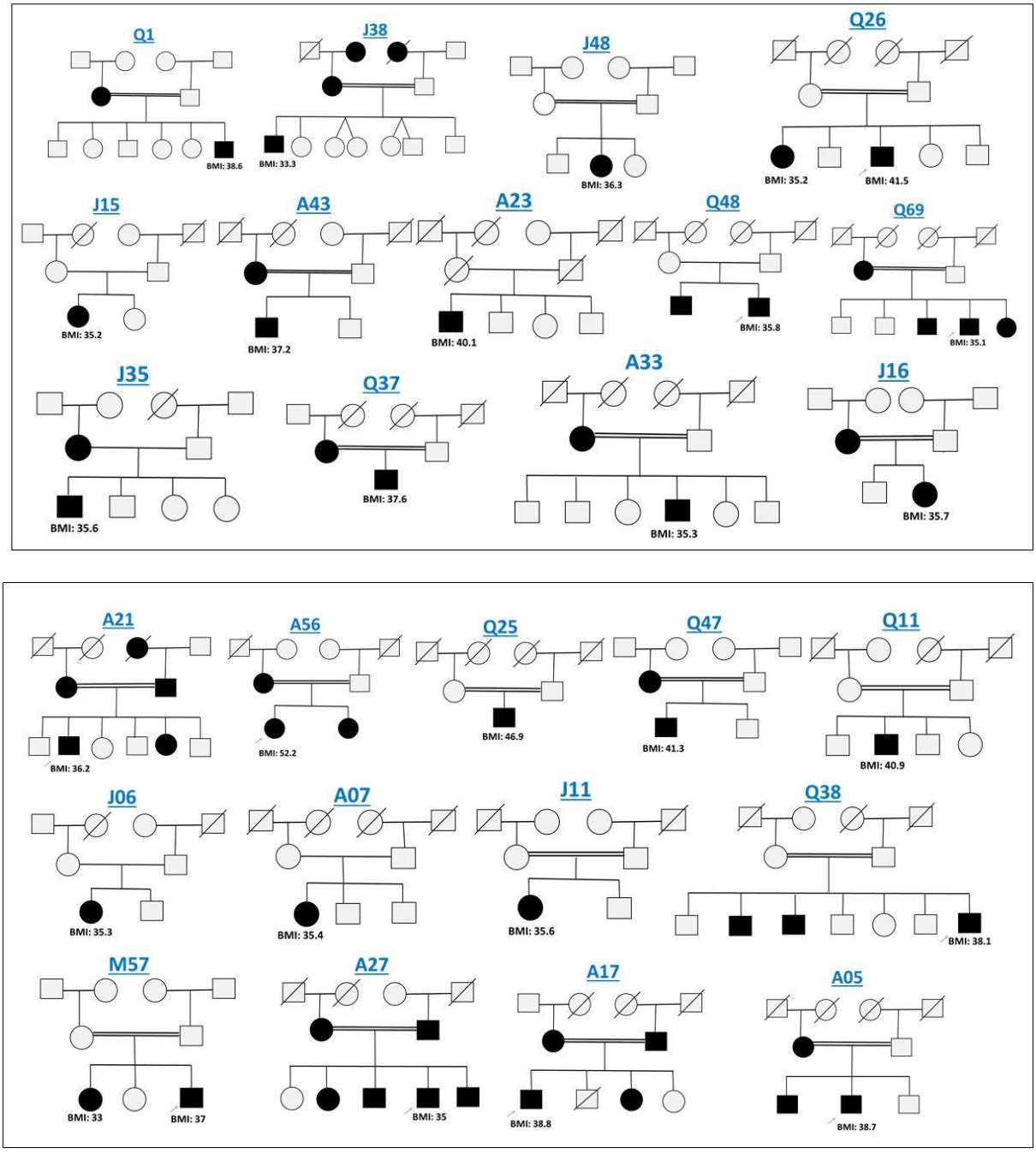
Supplementary Table 4: Rules for combining ACMG criteria to classify sequence variants as pathogenic or likely pathogenic

Pathogenic		(i)	1 Very Strong (PVS1) *AND* (a) ≥ 1 Strong (PS#) *OR* (b) ≥ 2 Moderate (PM#) *OR* (c) 1 Moderate (PM#) and 1 Supporting (PP#) *OR* (d) 2 Supporting (PP2 and PP3)
		(ii)	2 Strong (PS1 and PS3) *OR*
	PP3)	(iii)	1 Strong (PS#), 2 Moderate (PM#) and 2 Supporting (PP2 and
Likely pathogenic		(i)	1 Very Strong (PVS1) and 1 Moderate (PM#) *OR*
		(ii)	1 Strong (PS#) and 1-2 Moderate (PM#) *OR*
		(iii)	1 Strong and 2 Supporting (PP2 and PP3) *OR*
		(iv)	≥ 3 Moderate (PM#) *OR*
		(v)	2 Moderate (PM#) and 2 Supporting (PP2 and PP3)

Supplementary Table 5. Physical and biochemical characteristics of subjects carrying mutations in genes linked with monogenic obesity (SOPP – Young Adults cohort) (n = 26)

Characteristics	**Carriers**
Age (year)	19.4 ± 0.5[a]
Sex (M/F)	18 / 8
Height (cm)	165.7 ± 2.4
Weight (kg)	105.1 ± 3.6
BMI (kg/m²)	38.0 ± 0.8
Insulin (µIU/ml)	26.5 ± 4.3
Leptin (ng/ml)	33.8 ± 3.8
Cortisol (µg/dl)	18.2 ± 1.5

[a] Mean±SEM
F, female; M, male



Supplementary Figure 1. Family tree of probands with mutations in genes linked with monogenic obesity among the SOPP – Young Adults cohort

In case of probands Q26 and M57, access to samples from family members were available. In rest of the cases the obesity status indicated in the pedigrees is as reported at the time of recruitment.
A double line indicates first or second-degree cousin marriages