Blueprint Genetics

Monogenic Obesity Panel

Test code: KI1701

Is ideal for patients with a clinical suspicion of monogenic obesity

The panel covers genes associated with autosomal recessive and autosomal dominant forms of the disease and syndromes associated with obesity.

About Monogenic Obesity

Obesity is defined as abnormal or excessive fat accumulation that presents a risk to health, which occurs when abnormal amounts of triglycerides are stored in adipocytes and released as free fatty acids. In addition to dietary and lifestyle factors, epigenetic modifications play a role in excess fat accumulation. Obesity is correlated with an increased risk of type 2 diabetes, cardiovascular disease, cancer and mortality. The heritability of obesity and body weight in general is high. A small number of confirmed monogenic forms of obesity have been identified by molecular genetic studies. The identification of inborn deficiency of the mostly adipocyte-derived satiety hormone leptin in extremely obese children from consanguineous families paved the way to the first pharmacological therapy for obesity based on a molecular genetic finding. The Monogenic Obesity Panel includes syndromic conditions such as Bardet-Biedl syndrome, Cohen syndrome and Alström syndrome, where obesity is one feature in complex developmental disorders.

Availability

Results in 3-4 weeks

Gene set description

Genes in the Monogenic Obesity Panel and their clinical significance

Gene	Associated phenotypes	Inheritance	ClinVar	HGMD
ADCY3	Obesity	AR		6
ALMS1*	Alström syndrome	AR	197	302
ARL6	Bardet-Biedl syndrome, Retinitis pigmentosa	AR	14	21
BBS1	Bardet-Biedl syndrome	AR	66	103
BBS10	Bardet-Biedl syndrome	AR	90	107
BBS12	Bardet-Biedl syndrome	AR	36	58
BBS2	Bardet-Biedl syndrome, Retinitis pigmentosa	AR	58	91
BBS4	Bardet-Biedl syndrome	AR	25	53
BBS5	Bardet-Biedl syndrome	AR	18	31
BBS7	Bardet-Biedl syndrome	AR	19	43
BBS9	Bardet-Biedl syndrome	AR	27	52
CEP19	Morbid obesity and spermatogenic failure, Bardet-Biedl syndrome	AR	2	2

CEP290*	Bardet-Biedl syndrome, Leber congenital amaurosis, Joubert syndrome, Senior-Loken syndrome, Meckel syndrome	AR	130	289
CPE	Obesity, severe, and type II diabetes	AR		2
CUL4B	Mental retardation, syndromic, Cabezas	XL	23	38
DYRK1B	Abdominal obesity-metabolic syndrome	AD	2	2
GNAS	McCune-Albright syndrome, Progressive osseous heteroplasia, Pseudohypoparathyroidism, Albright hereditary osteodystrophy	AD	64	274
KSR2	Obesity	AD		28
LEP	Leptin deficiency	AR	5	20
LEPR	Leptin receptor deficiency	AR	4	30
MAGEL2	Schaaf-Yang syndrome (Prader-Willi-like syndrome)	AD	22	19
MC3R	Obesity due to MC3R deficiency	AD/AR		17
MC4R	Obesity	AD	37	152
MKKS	Bardet-Biedl syndrome, McKusick-Kaufman syndrome	AR	21	59
MKS1	Bardet-Biedl syndrome, Meckel syndrome	AR	50	52
NR0B2	Obesity, mild, early-onset	AD/AR	2	15
NTRK2	Obesity, hyperphagia, and developmental delay	AD	4	5
PCSK1	Proprotein convertase 1/3 deficiency	AD/AR	5	37
PHF6	Borjeson-Forssman-Lehmann syndrome	XL	22	29
PHIP	Obesity	AD	20	28
POMC	Proopiomelanocortin deficiency	AR	10	36
PPARG	Insulin resistance, Lipodystrophy, familial, partial	AD/Digenic (Severe digenic insulin resistance can be due to digenic mutations in PPP1R3A and PPARG)	19	49
SDCCAG8	Bardet-Biedl syndrome, Senior-Loken syndrome	AR	14	18
SH2B1	Obesity	AD	1	15
SIM1	6q16 deletion syndrome, Obesity due to SIM1 deficiency, Prader-Willi-like syndrome	AD/AR	2	44
TRIM32	Bardet-Biedl syndrome, Muscular dystrophy, limb-girdle	AR	13	16
TTC8	Bardet-Biedl syndrome, Retinitis pigmentosa	AR	5	16
TUB	Retinal dystrophy and obesity	AR	1	2

UCP3	Obesity, severe, and type II diabetes	AD/AR	2	6
VPS13B	Cohen syndrome	AR	351	203
WDPCP	Meckel-Gruber syndrome, modifier, Bardet-Biedl syndrome, Congenital heart defects, hamartomas of tongue, and polysyndactyly	AR	6	8

*Some regions of the gene are duplicated in the genome. Read more.

The gene has suboptimal coverage (means <90% of the gene's target nucleotides are covered at >20x with mapping quality score (MQ>20) reads), and/or the gene has exons listed under Test limitations section that are not included in the panel as they are not sufficiently covered with high quality sequence reads.

The sensitivity to detect variants may be limited in genes marked with an asterisk (*) or number sign (#)

Gene refers to the HGNC approved gene symbol; Inheritance refers to inheritance patterns such as autosomal dominant (AD), autosomal recessive (AR), mitochondrial (mi), X-linked (XL), X-linked dominant (XLD) and X-linked recessive (XLR); ClinVar refers to the number of variants in the gene classified as pathogenic or likely pathogenic in this database (ClinVar); HGMD refers to the number of variants with possible disease association in the gene listed in Human Gene Mutation Database (HGMD). The list of associated, gene specific phenotypes are generated from CGD or Mitomap databases.

Non-coding disease causing variants covered by the panel

Gene	Genomic location HG19	HGVS	RefSeq	RS-number
BBS1	Chr11:66291105	c.951+58C>T	NM_024649.4	
BBS4	Chr15:73001820	c.77-216delA	NM_033028.4	rs113994189
BBS5	Chr2:170354110	c.619-27T>G	NM_152384.2	
CEP290	Chr12:88462434	c.6012-12T>A	NM_025114.3	rs752197734
CEP290	Chr12:88494960	c.2991+1655A>G	NM_025114.3	rs281865192
CEP290	Chr12:88508350	c.1910-11T>G	NM_025114.3	
CEP290	Chr12:88534822	c.103-18_103-13delGCTTTT	NM_025114.3	
GNAS	Chr20:57478716	c.2242-11A>G	NM_080425.2	
MC4R	Chr18:58039645	c.-65_-64delTG	NM_005912.2	rs1255331292
PHIP	Chr6:79670165	c.3656+1242A>T	NM_017934.5	
POMC	Chr2:25387652	c.-11C>A	NM_000939.2	rs753856820
PPARG	Chr3:12421189	c.83-14A>G	NM_015869.4	

Test Strengths

The strengths of this test include:

- CAP accredited laboratory
- CLIA-certified personnel performing clinical testing in a CLIA-certified laboratory
- Powerful sequencing technologies, advanced target enrichment methods and precision bioinformatics pipelines

ensure superior analytical performance
- Careful construction of clinically effective and scientifically justified gene panels
- Some of the panels include the whole mitochondrial genome (please see the Panel Content section)
- Our Nucleus online portal providing transparent and easy access to quality and performance data at the patient level
- Our publicly available analytic validation demonstrating complete details of test performance
- ~2,000 non-coding disease causing variants in our clinical grade NGS assay for panels (please see 'Non-coding disease causing variants covered by this panel' in the Panel Content section)
- Our rigorous variant classification scheme
- Our systematic clinical interpretation workflow using proprietary software enabling accurate and traceable processing of NGS data
- Our comprehensive clinical statements

Test Limitations

The following exons are not included in the panel as they are not sufficiently covered with high quality sequence reads: *LEPR* (NM_001003680:20). Genes with suboptimal coverage in our assay are marked with number sign (#) and genes with partial, or whole gene, segmental duplications in the human genome are marked with an asterisk (*) if they overlap with the UCSC pseudogene regions. Gene is considered to have suboptimal coverage when >90% of the gene's target nucleotides are not covered at >20x with mapping quality score (MQ>20) reads. The technology may have limited sensitivity to detect variants in genes marked with these symbols (please see the Panel content table above).

This test does not detect the following:

- Complex inversions
- Gene conversions
- Balanced translocations
- Some of the panels include the whole mitochondrial genome but not all (please see the Panel Content section)
- Repeat expansion disorders unless specifically mentioned
- Non-coding variants deeper than ±20 base pairs from exon-intron boundary unless otherwise indicated (please see above Panel Content / non-coding variants covered by the panel).

This test may not reliably detect the following:

- Low level mosaicism in nuclear genes (variant with a minor allele fraction of 14.6% is detected with 90% probability)
- Stretches of mononucleotide repeats
- Low level heteroplasmy in mtDNA (>90% are detected at 5% level)
- Indels larger than 50bp
- Single exon deletions or duplications
- Variants within pseudogene regions/duplicated segments
- Some disease causing variants present in mtDNA are not detectable from blood, thus post-mitotic tissue such as skeletal muscle may be required for establishing molecular diagnosis.

The sensitivity of this test may be reduced if DNA is extracted by a laboratory other than Blueprint Genetics.

For additional information, please refer to the Test performance section and see our Analytic Validation.

Test performance

Our panels are sectioned from our high-quality, clinical grade NGS assay. Please see our sequencing and detection performance table for details regarding our ability to detect different types of alterations (Table).

Assays have been validated for various sample types including EDTA-blood, isolated DNA (excluding from formalin fixed paraffin embedded tissue), saliva and dry blood spots (filter cards). These sample types were selected in order to maximize the likelihood for high-quality DNA yield. The diagnostic yield varies depending on the assay used, referring healthcare professional, hospital and country. Plus analysis increases the likelihood of finding a genetic diagnosis for your patient, as large deletions and duplications cannot be detected using sequence analysis alone. Blueprint Genetics' Plus Analysis is a

combination of both sequencing and deletion/duplication (copy number variant (CNV)) analysis.

Performance of Blueprint Genetics high-quality, clinical grade NGS sequencing assay for panels.

	Sensitivity % (TP/(TP+FN)	Specificity %
Single nucleotide variants	99.89% (99,153/99,266)	>99.9999%
Insertions, deletions and indels by sequence analysis		
1-10 bps	99.2% (7,745/7,806)	>99.9999%
11-50 bps	99.13% (2,524/2,546)	>99.9999%
Copy number variants (exon level dels/dups)		
1 exon level deletion (heterozygous)	100% (20/20)	NA
1 exon level deletion (homozygous)	100% (5/5)	NA
1 exon level deletion (het or homo)	100% (25/25)	NA
2-7 exon level deletion (het or homo)	100% (44/44)	NA
1-9 exon level duplication (het or homo)	75% (6/8)	NA
Simulated CNV detection		
5 exons level deletion/duplication	98.7%	100.00%
Size range (0.1-47 Mb)	100% (25/25)	
The performance presented above reached by Blueprint Genetics high-quality, clinical grade NGS sequencing assay with the following coverage metrics		
Mean sequencing depth	143X	
Nucleotides with >20x sequencing coverage (%)	99.86%	

Performance of Blueprint Genetics Mitochondrial Sequencing Assay.

		Specificity
ANALYTIC VALIDATION (NA samples; n=4)		
Single nucleotide variants		
Heteroplasmic (45-100%)	100.0% (50/50)	100.0%
Heteroplasmic (35-45%)	100.0% (87/87)	100.0%

Heteroplasmic (25-35%)	100.0% (73/73)	100.0%
Heteroplasmic (15-25%)	100.0% (77/77)	100.0%
Heteroplasmic (10-15%)	100.0% (74/74)	100.0%
Heteroplasmic (5-10%)	100.0% (3/3)	100.0%
Heteroplasmic (<5%)	50.0% (2/4)	100.0%
CLINICAL VALIDATION (n=76 samples)		
All types		
Single nucleotide variants n=2084 SNVs		
Heteroplasmic (45-100%)	100.0% (1940/1940)	100.0%
Heteroplasmic (35-45%)	100.0% (4/4)	100.0%
Heteroplasmic (25-35%)	100.0% (3/3)	100.0%
Heteroplasmic (15-25%)	100.0% (3/3)	100.0%
Heteroplasmic (10-15%)	100.0% (9/9)	100.0%
Heteroplasmic (5-10%)	92.9%(12/13)	99.98%
Heteroplasmic (<5%)	88.7% (47/53)	99.93%
Insertions and deletions by sequence analysis n=42 indels		
Heteroplasmic (45-100%) 1-10bp	100.0% (32/32)	100.0%
Heteroplasmic (5-45%) 1-10bp	100.0% (3/3)	100.0%
Heteroplasmic (<5%) 1-10bp	100.0% (5/5)	>0.9999
SIMULATION DATA /(mitomap mutations)		
Insertions, and deletions 1-24 bps by sequence analysis; n=17		
Homoplasmic (100%) 1-24bp	100.0% (17/17)	99.98%
Heteroplasmic (50%)	100.0% (17/17)	99.99%
Heteroplasmic (25%)	100.0% (17/17)	100.0%
Heteroplasmic (20%)	100.0% (17/17)	100.0%
Heteroplasmic (15%)	100.0% (17/17)	100.0%
Heteroplasmic (10%)	94.1% (16/17)	100.0%
Heteroplasmic (5%)	94.1% (16/17)	100.0%
Copy number variants (separate artifical mutations; n=1500)		
Homoplasmic (100%) 500 bp, 1kb, 5 kb	100.0%	100.0%
Heteroplasmic (50%) 500 bp, 1kb, 5 kb	100.0%	100.0%

Heteroplasmic (30%) 500 bp, 1kb, 5 kb	100.0%	100.0%
Heteroplasmic (20%) 500 bp, 1kb, 5 kb	99.7%	100.0%
Heteroplasmic (10%) 500 bp, 1kb, 5 kb	99.0%	100.0%
The performance presented above reached by following coverage metrics at assay level (n=66)		
	Mean of medians	Median of medians
Mean sequencing depth MQ0 (clinical)	18224X	17366X
Nucleotides with >1000x MQ0 sequencing coverage (%) (clinical)	100%	
rho zero cell line (=no mtDNA), mean sequencing depth	12X	

Bioinformatics

The target region for each gene includes coding exons and ±20 base pairs from the exon-intron boundary. In addition, the panel includes non-coding and regulatory variants if listed above (Non-coding variants covered by the panel). Some regions of the gene(s) may be removed from the panel if specifically mentioned in the 'Test limitations" section above. The sequencing data generated in our laboratory is analyzed with our proprietary data analysis and annotation pipeline, integrating state-of-the art algorithms and industry-standard software solutions. Incorporation of rigorous quality control steps throughout the workflow of the pipeline ensures the consistency, validity and accuracy of results. Our pipeline is streamlined to maximize sensitivity without sacrificing specificity. We have incorporated a number of reference population databases and mutation databases including, but not limited, to 1000 Genomes Project, gnomAD, ClinVar and HGMD into our clinical interpretation software to make the process effective and efficient. For missense variants, *in silico* variant prediction tools such as SIFT, PolyPhen, MutationTaster are used to assist with variant classification. Through our online ordering and statement reporting system, Nucleus, ordering providers have access to the details of the analysis, including patient specific sequencing metrics, a gene level coverage plot and a list of regions with <20X sequencing depth if applicable. This reflects our mission to build fully transparent diagnostics where ordering providers can easily visualize the crucial details of the analysis process.

Clinical interpretation

We provide customers with the most comprehensive clinical report available on the market. Clinical interpretation requires a fundamental understanding of clinical genetics and genetic principles. At Blueprint Genetics, our PhD molecular geneticists, medical geneticists and clinical consultants prepare the clinical statement together by evaluating the identified variants in the context of the phenotypic information provided in the requisition form. Our goal is to provide clinically meaningful statements that are understandable for all medical professionals regardless of whether they have formal training in genetics.

Variant classification is the corner stone of clinical interpretation and resulting patient management decisions. Our classifications follow the Blueprint Genetics Variant Classification Schemes based on the ACMG guideline 2015. Minor modifications were made to increase reproducibility of the variant classification and improve the clinical validity of the report. Our experience with tens of thousands of clinical cases analyzed at our laboratory allowed us to further develop the industry standard.

The final step in the analysis is orthogonal confirmation. Sequence variants classified as pathogenic, likely pathogenic and variants of uncertain significance (VUS) are confirmed using bi-directional Sanger sequencing when they do not meet our stringent NGS quality metrics for a true positive call. Reported heterozygous and homo/hemizygous copy number variations with a size <10 and <3 target exons are confirmed by orthogonal methods such as qPCR if the specific CNV has been seen and confirmed less than three times at Blueprint Genetics.

Our clinical statement includes tables for sequencing and copy number variants that include basic variant information (genomic coordinates, HGVS nomenclature, zygosity, allele frequencies, in silico predictions, OMIM phenotypes and classification of the variant). In addition, the statement includes detailed descriptions of the variant, gene and phenotype(s) including the role of the specific gene in human disease, the mutation profile, information about the gene's variation in

population cohorts and detailed information about related phenotypes. We also provide links to the references, abstracts and variant databases used to help ordering providers further evaluate the reported findings if desired. The conclusion summarizes all of the existing information and provides our rationale for the classification of the variant.

Identification of pathogenic or likely pathogenic variants in dominant disorders or their combinations in different alleles in recessive disorders are considered molecular confirmation of the clinical diagnosis. In these cases, family member testing can be used for risk stratification. We do not recommend using variants of uncertain significance (VUS) for family member risk stratification or patient management. Genetic counseling is recommended.

Our interpretation team analyzes millions of variants from thousands of individuals with rare diseases. Our internal database and our understanding of variants and related phenotypes increases with every case analyzed. Our laboratory is therefore well-positioned to re-classify previously reported variants as new information becomes available. If a variant previously reported by Blueprint Genetics is re-classified, our laboratory will issue a follow-up statement to the original ordering health care provider at no additional cost.

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ICD codes

Commonly used ICD-10 codes when ordering the Monogenic Obesity Panel

ICD-10	Disease
E66.9	Monogenic obesity

Accepted sample types

- EDTA blood, min. 1 ml
- Purified DNA, min. 3µg*
- Saliva (Oragene DNA OG-500 kit)

Label the sample tube with your patient's name, date of birth and the date of sample collection.

Note that we do not accept DNA samples isolated from formalin-fixed paraffin-embedded (FFPE) tissue.

Resources

- Alström Syndrome International
- Alström Syndrome UK
- Bardet Biedl Syndrome Family Association
- Bardet-Biedl Australia
- Clinical Registry Investigating Bardet-Biedl Syndrome
- Clinical Registry Investigating Bardet-Biedl Syndrome
- Cohen syndrome Association
- Foundation for Prader-Willi Research
- GeneReviews - Alström Syndrome
- GeneReviews - Bardet-Biedl Syndrome
- GeneReviews - Cohen syndrome
- GeneReviews - Prader-Willi Syndrome
- International Prader-Willi Syndrome Organisation
- Kaur Y, et al. A systematic review of genetic syndromes with obesity. Obes Rev. 2017 Jun;18(6):603-634.
- NORD - Alström Syndrome
- NORD - Bardet-Biedl Syndrome
- NORD - Cohen Syndrome

- NORD - Prader-Willi Syndrome
- Obesity Medicine Association
- Prader-Willi Syndrome Association
- Rhythm Pharmaceuticals – The Genetic Obesity Program
- Ryan DH & Kahan S, Guideline Recommendations forObesityManagement. Med Clin North Am. 2018 Jan;102(1):49-63.