THE UNIVERSITY OF CHICAGO | Genetic Services Laboratories

Next Generation Sequencing Panels for Lipodystrophy

Lipodystrophies are characterized by generalized or partial absence of adipose tissue and are typically considered in individuals with insulin resistance, significant dyslipidaemia and fatty liver. Lipodystrophies are typically classified according to the anatomical distribution of fat tissue:

Congenital generalized lipodystrophy, which is typically apparent from birth, is characterized by generalized loss of adipose tissue affecting the limbs, trunk, face and neck. Advanced bone age and linear growth and skeletal muscle prominence can be seen during childhood. Severe dyslipidaemia, hepatomegaly and non-alcoholic steatohepatitis are almost always noted.

Partial lipodystrophy, which may not be prominent until puberty and is typically milder, is characterized by abnormal fat topography along with an overall reduction in fat mass affecting the limb with variable truncal involvement and normal or excess fat on the face and neck. Women are typically more severely affected than men. Asymptomatic impaired glucose tolerance to severe insulin resistance can be noted, and non-alcoholic steatohepatitis and cardiovascular disease are common complications.

Our Comprehensive Lipodystrophy Panel includes mutation analysis of the 19 genes listed below.
Our Partial Lipodystrophy Panel includes mutation analysis of the 13 genes listed below.
Our Congenital Generalized Lipodystrophy Panel includes mutation analysis of the 7 genes listed below.

Congenital Generalized Lipodystrophy		Partial Lipodystrophy			Comprehensive Lipodystrophy			
AGPAT2	PCYT1A	ADRA2A	LMNA	PSMB8	ADRA2A	CIDEC	LMNB2	PSMB8
BSCL2	PTRF	AKT2	LMNB2	TBC1D4	AGPAT2	FBN1	PCYT1A	PTRF
CAV1		CAV1	PIK3R1	ZMPSTE24	AKT2	KCNJ6	PIK3R1	TBC1D4
FBN1		CIDEC	POLD1		BSCL2	LIPE	POLD1	ZMPSTE24
KCNJ6		LIPE	PPARG		CAV1	LMNA	PPARG	

Congenital Lipodystrophy genes		
Gene	**Inheritance**	**Disease Phenotype and Molecular Genetics**
AGPAT2 [OMIM# 603100]	AR	Homozygous or compound heterozygous mutations in *AGPAT2*, encoding the enzyme acylglycerol-3-phosphate O -acyltransferase 2 (AGPAT2), cause autosomal recessive Berardinelli-Seip congenital lipodystrophy (CGL) type 1 [1]. This acyltransferase enzyme, located in the ER, catalyses the conversion of lysophosphatidic acid to phosphatidic acid, a key step in the synthesis of triglycerides and glycerophospholipids from glycerol-3-phosphate. Collectively, mutations in *AGPAT2* and *BSCL2* account for over 95% of cases of CGL.
BSCL2 [OMIM#606158]	AR	Homozygous or compound heterozygous mutations in *BSCL2* encoding seipin, a protein of unknown function localized to the endoplasmic reticulum membrane, cause autosomal recessive Berardinelli-Seip congenital lipodystrophy (CGL) type 2 [2]. Patients with lipodystrophy resulting from *BSCL2* mutations are difficult to reliably distinguish from *AGPAT2* mutations on clinical grounds alone, although the loss of adipose tissue from mechanical fat pads such as the palms, soles, orbits, scalp and periarticular regions may be specific to BSCL2 mutations and may indicate a distinct pathological mechanism [3].
CAV1 [OMIM#601047]	AR	A single patient with generalized lipodystrophy and short stature (CGL type 3) has been identified with a homozygous loss-of-function mutation in *CAV1*, encoding caveolin-1, one of a number of proteins mediating caveola formation [4]. Caveolae are specialized plasma membrane microdomains involved in numerous processes, including signal transduction and and lipid trafficking [5]. Heterozygous *CAV1* mutations have been identified in individuals with partial lipodystrophy (see partial lipodystrophy summary below).
FBN1 [OMIM# 134797]	AD	Truncating variants in exon 64 of the *FBN1* gene have been associated with patients with marfanoid features, and congenital lipodystrophy with a neonatal progeroid appearance [6]. Typical facial features include proptosis, downslanting palpebral fissures, and retrognathia. Features that overlap with classic Marfan syndrome are also typically present, including including arachnodactyly, digital hyperextensibility, myopia, dural ectasia, and normal psychomotor development [6].

KCNJ6 [OMIM# 600877]		Heterozygous mutations in the KCNJ6 gene have been associated with Keppen-Lubinsky syndrome, a rare condition associated with generalized lipodystrophy, severe developmental delay, hypertonia, hyperreflexia, microcephaly, prominent nasal bridge and open mouth [7].
PCYT1A [OMIM# 123695]	AR	Payne *et al.*, 2014, identified biallelic loss of function variants in *PCYT1A* in two unrelated patients with lipodystrophy, severe insulin resistance, and diabetes, in addition to severe fatty liver disease and low HDL cholesterol levels [8]. Functional analyses using lymphocytes and fibroblasts from the affected individuals showed that the PRYT1A protein was barely detectable [8].
PTRF [OMIM# 603198]	AR	Homozygous or compound heterozygous mutations in *PTRF* encoding cavin, an essential factor in the biogenesis of caveolae, have recently been identified in several kindreds with an autosomal recessive disorder characterized by generalised lipodystrophy and muscular dystrophy (CGL type 4) [9,10].

Partial Lipodystrophy genes		
Gene	**Inheritance**	**Disease Phenotype and Molecular Genetics**
ADRA2A [OMIM#104210]	AD	A missense mutation in *ADRA2A* was identified in an African American patient with atypical familial partial lipodystrophy. The mutation segregated with affected individuals within this kindred. Functional studies demonstrated that this variant leads to decreased suppression of cAMP in HEK-293 cells and increased lipolysis in adipocytes, suggesting a loss-of-function effect [11].
AKT2 [OMIM#164731]	AD	Missense mutations in the *AKT2* gene have been identified in patients with hypoinsulinemic hypoglycemia with hemihypertrophy [12] and dyslipidemia and hepatic steatosis [13].
CAV1 [OMIM# 601047]	AD	A heterozygous frameshift mutation in *CAV1* has been identified in a father and daughter with partial lipodystrophy, congenital cataracts and neurodegeneration syndrome. An additional individual with partial lipodystrophy and congenital cataracts but no apparent neurologic involvement has also been found to have a heterozygous mutation in the *CAV1* gene [14].
CIDEC [OMIM#612120]	AR	Recently, a homozygous mutation in *CIDEC*, encoding a lipid droplet-associated protein, was identified in a patient with partial lipodystrophy (affecting limb, femorogluteal and subcutaneous abdominal fat), white adipocytes with multiloculated lipid droplets and insulin-resistant diabetes [15].
LIPE [OMIM# 615980]	AR	A homozygous mutation in the *LIPE* gene has been associated with late onset partial lipodystrophy in a consanguineous Italian family [16].
LMNA [OMIM#150330]	AD	Autosomal dominant mutations in *LMNA*, encoding lamin A/C, cause Dunnigan-type familial partial lipodystrophy type 2 (FPLD2) [17,18]. Children are born with an apparently normal fat distribution but typically manifest clinically discernible limb and truncal lipodystrophy with the onset of puberty (when fat depots normally expand). Fat around the head and neck, and visceral fat are typically spared. Metabolic abnormalities include insulin-resistant diabetes mellitus with acanthosis nigricans and hypertriglyceridemia; hirsutism and menstrual abnormalities occur infrequently.
LMNB2 [OMIM# 608709]	AD	Variants in the *LMNB2* gene may increase susceptibility to acquired partial lipodystrophy, characterized by gradual onset of bilaterally symmetrical loss of subcutaneous fat from the face, neck, upper extremities, thorax, and abdomen [19]. Affected individuals may have other factors that increase susceptibility to lipodystrophy, including other genetic and environmental factors [19]. Females are more likely to be affected by acquired lipodystrophy in males.
PIK3R1 [OMIM# 269880]	AD	Heterozygous variants in the *PIK3R1* gene are associated with SHORT syndrome, which is characterized by short stature, hyperextensibility, ocular depression, Rieger anomaly, and teething delay [20]. Affected individuals typically also have partial lipodystrophy of the face and upper limbs, and insulin resistance [20].
POLD1 [OMIM# 615381]	AD	Mutations in the *POLD1* gene are aassociated with mandibular hypoplasia, deafness, progeroid features and lipodystrophy (MDPL) syndrome [21].
PPARG [OMIM#601487]	AD	Loss-of-function mutations in the nuclear hormone receptor peroxisome proliferator-activated receptor gamma, *PPARG,* cause familial partial lipodystrophy type 3 (FPLD3) [22]. The lipodystrophy is characterised by distal lipoatrophy, but facial adipose tissue may be increased, decreased or normal. Diabetes, hypertension and hypertriglyceridaemia are also present. The metabolic anomalies, rather than the lipoatrophy, are the most marked and prominent features of the disease.
PSMB8	AR	Biallelic mutations in the *PSMB8* gene is associated with autoinflammation, lipodystrophy and dermatosis syndrome [23].

TBC1D4 [OMIM#612465]	AD	Mutations in *TBC1D4*, *a* major effector of insulin signaling in pancreatic beta cells, have been identified in patients with acanthosis nigricans and extreme postprandial hyperinsulinemia [24].
ZMPSTE24 [OMIM#606480]	AR	Compound heterozygous mutations in the ZMPSTE24 gene, encoding a metalloproteinase essential for the processing of prelamin A to the mature lamin A protein, cause a syndrome of partial lipodystrophy with mandibuloacral dysplasia (MAD) [25].

Test methods:
Comprehensive sequence coverage of the coding regions and splice junctions of all genes in this panel will be performed. Targets of interests will be captured and amplified using the Agilent SureSelect system. The constructed genomic DNA library will be sequenced using Illumina technology and reads will be aligned to the reference sequence. Variants will be identified and evaluated using a custom collection of bioinformatic tools and comprehensively interpreted by our team of directors and genetic counselors. All pathogenic and likely pathogenic variants will be confirmed by Sanger sequencing. The technical sensitivity of this test is estimated to be >99% for single nucleotide changes and insertions and deletions of less than 20bp. Our CNV detection algorithm was developed and its performance determined for the sole purpose of identifying deletions and duplications within the coding region of the gene(s) tested. Partial exonic copy number changes and rearrangements of less than 400 bp may not be detected by this methodology. Regions of high homology and repetitive regions may not be analyzed. This methodology will not detect low level mosaicism, balanced translocations, inversions, or point mutations that may be responsible for the clinical phenotype. The sensitivity of our deletion/duplication assay may be reduced when DNA extracted by an outside laboratory is provided.

Comprehensive Lipodystrophy Panel (19 genes)

Sample specifications:	3 to10 cc of blood in a purple top (EDTA) tube
Cost:	$3000
CPT codes:	81406
	81407
Turn-around time:	8 weeks

Note: We cannot bill insurance for this test

Partial Lipodystrophy Panel (13 genes)

Sample specifications:	3 to10 cc of blood in a purple top (EDTA) tube
Cost:	$3000
CPT codes:	81406
	81407
Turn-around time:	8 weeks

Note: We cannot bill insurance for this test

Congenital Generalized Lipodystrophy Panel (7 genes)

Sample specifications:	3 to10 cc of blood in a purple top (EDTA) tube
Cost:	$2000
CPT codes:	81406
	81407
Turn-around time:	8 weeks

Note: We cannot bill insurance for this test

Results:
Results, along with an interpretive report, are faxed to the referring physician as soon as they are completed. One report will be issued for the entire panel. All abnormal results are reported by telephone.

For more information about our testing options, please visit our website at dnatesting.uchicago.edu or contact us at 773-834-0555.

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