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CLINICAL UTILITY GENE CARD

Clinical Utility Gene Card for: Congenital Generalized Lipodystrophy

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1. DISEASE CHARACTERISTICS

1.1 Name of the disease (synonyms)

There are four subclasses of Congenital Generalized Lipodystrophy (CGL), also named Berardinelli–Seip Congenital Lipodystrophy (BSCL):

Type 1 CGL (CGL1).
Type 2 CGL (CGL2).
Type 3 CGL (CGL3).
Type 4 CGL (CGL4).

It is important to underline that CGL is an expanding group of disorders, whose classification is still underway. In addition to the diseases known as CGL in OMIM, generalized lipodystrophies can be encountered in other Mendelian disorders including severe insulin-resistance syndromes, complex progeroid syndromes, as well as autoinflammatory diseases. A *de novo* variant in the promoter of the *FOS* gene has also been reported in a generalized lipodystrophy, though this disease has not entered the CGL classification so far.[1] The description of all these rare entities is beyond the scope of this clinical utility gene card, which will focus on the so-called CGL.

1.2 OMIM# of the disease

CGL1: #608594.
CGL2: #269700.
CGL3: #612526.
CGL4: #613327.

1.3 Name of the analysed genes or dna/chromosome segments

CGL1: *AGPAT2*.
CGL2: *BSCL2*.
CGL3: *CAV1*.
CGL4: *PTRF*.

1.4 OMIM# of the gene(s)

AGPAT2: *603100.
BSCL2: *606158.
CAV1: *601047.
PTRF: *603198.

1.5 Mutational spectrum

AGPAT2—CGL1 is an autosomal recessive disorder. Several dozen disease-causing variants have been described.[2] The following molecular defects have been reported: nonsense, missense, splice-site variants, deletions and insertions.[3] Most of them result in frameshift and/or truncated proteins, which are likely to lead to the complete loss of AGPAT2 function. This can be demonstrated *in vitro* by measurement of AGPAT2 enzymatic activity.[4]

BSCL2—CGL2 is an autosomal recessive disorder. Several dozen disease-causing variants have been described. The following molecular defects have been reported: nonsense, missense, splice-site variants, deletions and insertions.[3,5] Most of them result in frameshift and/or truncated proteins, which are likely to lead to complete loss of BSCL2 function (for more details, see http://databases.lovd.nl/shared/variants/BSCL2/unique). Notably, disease-causing variants in this gene have also been identified in patients with distal hereditary motor neuropathies or neurodegenerative syndromes.[6]

CAV1—disease-causing variants in this gene are very rare. A homozygous nonsense variant was identified in a patient with CGL.[7] In addition, a nonsense variant, two deletions leading to frameshifts and a variant located within the 5′-untranslated region of the gene were reported in the heterozygous state in patients with atypical forms of lipodystrophies, either partial or generalized.[8–10] Notably, disease-causing variants in this gene have also been identified in patients described with isolated pulmonary hypertension.[11]

PTRF—CGL4 is an autosomal recessive disorder associating generalized lipodystrophy and muscular dystrophy. A bit more than 10 disease-causing variants have been described to date. The following molecular defects have been reported: nonsense, splice-site variants, deletions and insertions.[12] All of them result in frameshift and/or truncated proteins, which are likely to lead to complete loss of PTRF function. Individuals carrying disease-causing variants in the heterozygous state can present minor signs of the disease.

Except for *BSCL2*, there is no specific database listing molecular defects implicated in CGL.

1.6 Analytical methods

Sanger sequencing of PCR products corresponding to the coding regions and conserved splice sites is performed on a routine basis. Next-generation sequencing, including gene-targeted and whole-exome sequencing approaches, is also used.

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1.7 Analytical validation

There are several steps in the analytical validation process.

- Sequencing of both DNA strands (forward and reverse) is performed.
- When two heterozygous variants or a homozygous variant are found, testing of the patients' parents is recommended to confirm that the defect is biallelic. More generally, identification of the same variant in the affected proband's relatives provides additional confirmation of the result. When the genetic test is positive, a search of the molecular defects is also recommended on a second independent sample from the patient.
- Newly discovered variants may be searched for in databases listing benign and pathogenic variants. Pathogenicity of variants can also be tested by *in silico* prediction methods and functional studies.

Notably, there is to date no external quality assessment dedicated to this specific set of genes proposed by the European Molecular Genetics Quality Network.

1.8 Estimated frequency of the disease (Incidence at birth ('birth prevalence') or population prevalence. If known to be variable between ethnic groups, please report):

Less than 500 patients have been reported worldwide. The population prevalence has been estimated to be about 1 in 10 million.[13]

1.9 Diagnostic setting

	Yes	No
A. (Differential) diagnostics	☒	☐
B. Predictive testing	☐	☒
C. Risk assessment in relatives	☒	☐
D. Prenatal	☒	☐

Comment:

CGL is characterized by a loss of nearly all the body fat with extreme muscularity. Manifestations appear at birth or during early infancy and are associated with metabolic complications (insulin resistance with acanthosis nigricans and alterations of glucose homeostasis, hypertriglyceridaemia and hepatic steatosis).

2. TEST CHARACTERISTICS

	Genotype or disease		A: True positives B: False positives	C: False negative D: True negative
	Present	Absent		
Test				
Positive	A	B	Sensitivity:	A/(A+C)
			Specificity:	D/(D+B)
Negative	C	D	Positive predictive value:	A/(A+B)
			Negative predictive value:	D/(C+D)

2.1 Analytical sensitivity
(**proportion of positive tests if the genotype is present**)

Depending on the quality of sequencing methods, the analytical sensitivity is close to 100% for germline variants located in coding regions and flanking intronic sequences. Single-nucleotide polymorphisms (SNPs) within PCR primer-binding sites can result in preferential amplification of a single allele and constitute a rare cause of missed variant, so that careful checking of primer-binding sites for SNPs is essential. Notably, potential deep-intronic variants, variants in promoters, large deletions and duplications would not be detected by Sanger sequencing performed on a routine basis. Next-generation sequencing allows the detection of copy-number variations.

2.2 Analytical specificity
(**proportion of negative tests if the genotype is not present**)

100%. The analytical validation described above should avoid false-positive tests. As CGL are recessive disorders, false-positive results with two unknown sequence variations are not expected.

2.3 Clinical sensitivity
(**proportion of positive tests if the disease is present**)

The clinical sensitivity can be dependent on variable factors such as age, sex or family history. In such cases a general statement should be given, even if a quantification can only be made case by case.

When the diagnosis has been properly established based on clinical investigation, family history, imaging and biochemical results, very few negative tests are expected. Molecular testing of *AGPAT2* and *BSCL2* explain more than 95% of cases,[3] whereas *CAV1* and *PTRF* explain very few of them. When genetic testing is negative in a patient with symptoms evocative of CGL, differential diagnoses can be considered (please see section 3.1).

2.4 Clinical specificity
(**proportion of negative tests if the disease is present**)

The clinical specificity can be dependent on variable factors such as age or family history. In such cases a general statement should be given, even if a quantification can only be made case by case.

Close to 100%. A precise quantification is difficult, since molecular testing of CGL genes is not performed on a routine basis in asymptomatic individuals.

2.5 Positive clinical predictive value
(life time risk to develop the disease if the test is positive)

The positive clinical predictive value is 100%. Incomplete penetrance is extremely rare in autosomal recessive disorders and has not been reported in CGL. As mentioned previously, disease onset is at birth or during early childhood.

2.6 Negative clinical predictive value
(probability of not developing the disease if the test is negative)

Assume an increased risk based on family history for a non-affected person. Allelic and locus heterogeneity may need to be considered.

Index case in that family had been tested:

The negative clinical predictive value is nearly 100%, although a negative test does not exclude the possibility of developing a CGL due to molecular defects in other genes that were not tested.

Index case in that family had not been tested:

Genetic testing for a clinically unaffected individual is not indicated in this situation. It would only be undertaken if a variant in a gene responsible for CGL has been identified in the proband.

3. CLINICAL UTILITY

3.1 (Differential) diagnostics: The tested person is clinically affected
(To be answered if in 1.9 'A' was marked)

3.1.1 Can a diagnosis be made other than through a genetic test?

No	☐ (continue with 3.1.4)	
Yes	☒	
	Clinically	☒
	Imaging	☒
	Endoscopy	☐
	Biochemistry	☒
	Electrophysiology	☐
	Other (please describe)	

Genetic testing helps to confirm the clinical diagnosis. Indeed, CGL shares clinical features with acquired generalized lipodystrophy, severe insulin-resistance syndromes (such as Rabson–Mendenhall syndrome, Donohue syndrome or SHORT syndrome), atypical progeroid syndromes and autoinflammatory diseases.[14,15]

3.1.2 Describe the burden of alternative diagnostic methods to the patient

In typical cases, clinical diagnosis is strongly suggested by combining family history, physical examination, biochemical results and imagery. There are no invasive procedures for the patient.

3.1.3 How is the cost effectiveness of alternative diagnostic methods to be judged?

Clinical investigations, biochemical assays and imagery are actually used to get an accurate clinical evaluation of the patients, which is necessary for their proper management and follow-up. This does not exclude genetic testing and vice versa. Both diagnostic procedures add to the global picture.

3.1.4 Will disease management be influenced by the result of a genetic test?

No	☐	
Yes	☒	
	Therapy (please describe)	Whatever the result of the genetic test, treatment of CGL is mainly directed towards the metabolic alterations that are apparent in each individual. In addition to non-specific therapies, the use of recombinant leptin can be considered. This treatment is approved in the United States and in Japan for CGL, but is only available through compassionate programs in Europe.
	Prognosis (please describe)	Genetic testing helps to predict the future course of the disease, as well as the risk of complications (mainly at the metabolic, cardiovascular, bone and gastrointestinal levels). Molecular defects in certain genes are more frequently associated with particular symptoms (eg, among others, *AGPAT2* with bone cysts, *BSCL2* with mental retardation, *CAV1* with hypocalcaemia and *PTRF* with muscular dystrophy). Knowledge of the genetic cause should lead healthcare providers to customize surveillance for complications, and may inform specific therapeutic approaches. Genetic testing is also crucial for prenatal diagnosis and genetic counseling.
	Management (please describe)	Because CGL are multisystem disorders, follow-up by a multidisciplinary team is important (pediatricians, endocrinologists, cardiologists, nutritionists and surgeons) and regular surveillance and therapeutic management are mandatory. Reconstructive surgery can be proposed in adults. Special education is required for individuals with intellectual disability.

3.2 Predictive Setting: The tested person is clinically unaffected but carries an increased risk based on family history

(To be answered if in 1.9 'B' was marked)

3.2.1 Will the result of a genetic test influence lifestyle and prevention?

If the test result is **positive** (please describe):
Not applicable.
If the test result is **negative** (please describe):
Not applicable.

3.2.2 Which options in view of lifestyle and prevention does a person at-risk have if no genetic test has been done (please describe)?

Not applicable.

3.3 Genetic risk assessment in family members of a diseased person

(To be answered if in 1.9 'C' was marked)

3.3.1 Does the result of a genetic test resolve the genetic situation in that family?

Usually yes. A positive test in a patient may lead, at adult age, to test the carriership of his/her partner.

3.3.2 Can a genetic test in the index patient save genetic or other tests in family members?

Yes.

3.3.3 Does a positive genetic test result in the index patient enable a predictive test in a family member?

No. Because CGL appears with striking manifestations, screening family members who do not display any feature of the phenotype is unnecessary.

3.4 Prenatal diagnosis

(To be answered if in 1.9 'D' was marked)

3.4.1 Does a positive genetic test result in the index patient enable a prenatal diagnosis?

Yes, prenatal diagnosis can be performed for parents having an affected child. It can also be proposed in the offspring of couples, in which each member carries at least one variant.

4. IF APPLICABLE, FURTHER CONSEQUENCES OF TESTING

Please assume that the result of a genetic test has no immediate medical consequences. Is there any evidence that a genetic test is nevertheless useful for the patient or his/her relatives? (Please describe).

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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1 Knebel B, Kotzka J, Lehr S *et al*: A mutation in the c-fos gene associated with congenital generalized lipodystrophy. *Orphanet J Rare Dis* 2013; **8**: 119.
2 Miranda DM, Wajchenberg BL, Calsolari MR *et al*: Novel mutations of the BSCL2 and AGPAT2 genes in 10 families with Berardinelli-Seip congenital generalized lipodystrophy syndrome. *Clin Endocrinol* 2009; **71**: 512–517.
3 Agarwal AK, Simha V, Oral EA *et al*: Phenotypic and genetic heterogeneity in congenital generalized lipodystrophy. *J Clin Endocrinol Metab* 2003; **88**: 4840–4847.
4 Haque W, Garg A, Agarwal AK: Enzymatic activity of naturally occurring 1-acylglycerol-3-phosphate-O-acyltransferase 2 mutants associated with congenital generalized lipodystrophy. *Biochem Biophys Res Commun* 2005; **327**: 446–453.
5 Magré J, Délépine M, Khallouf E *et al*: Identification of the gene altered in Berardinelli-Seip congenital lipodystrophy on chromosome 11q13. *Nat Genet* 2001; **28**: 365–370.
6 Guillén-Navarro E, Sánchez-Iglesias S, Domingo-Jiménez R *et al*: A new seipin-associated neurodegenerative syndrome. *J Med Genet* 2013; **50**: 401–409.
7 Kim CA, Délépine M, Boutet E *et al*: Association of a homozygous nonsense caveolin-1 mutation with Berardinelli-Seip congenital lipodystrophy. *J Clin Endocrinol Metab* 2008; **93**: 1129–1134.
8 Cao H, Alston L, Ruschman J, Hegele RA: Heterozygous CAV1 frameshift mutations (MIM 601047) in patients with atypical partial lipodystrophy and hypertriglyceridemia. *Lipids Health Dis* 2008; **7**: 3.
9 Garg A, Kircher M, Del Campo M, Amato RS, Agarwal AK: Whole exome sequencing identifies *de novo* heterozygous CAV1 mutations associated with a novel neonatal onset lipodystrophy syndrome. *Am J Med Genet A* 2015; **167A**: 1796–1806.
10 Schrauwen I, Szelinger S, Siniard AL *et al*: A frame-shift mutation in CAV1 is associated with a severe neonatal progeroid and lipodystrophy syndrome. *PLoS One* 2015; **10**: e0131797.
11 Austin ED, Ma L, LeDuc C *et al*: Whole exome sequencing to identify a novel gene (caveolin-1) associated with human pulmonary arterial hypertension. *Circ Cardiovasc Genet* 2012; **5**: 336–343.
12 Jelani M, Ahmed S, Almramhi MM *et al*: Novel nonsense mutation in the PTRF gene underlies congenital generalized lipodystrophy in a consanguineous Saudi family. *Eur J Med Genet* 2015; **58**: 216–221.
13 Garg A: Acquired and inherited lipodystrophies. *N Engl J Med* 2004; **350**: 1220–1234.
14 Robbins AL, Savage DB: The genetics of lipid storage and human lipodystrophies. *Trends Mol Med* 2015; **21**: 433–438.
15 Vigouroux C, Caron-Debarle M, Le Dour C, Magré J, Capeau J: Molecular mechanisms of human lipodystrophies: from adipocyte lipid droplet to oxidative stress and lipotoxicity. *Int J Biochem Cell Biol* 2011; **43**: 862–876.