Caveolar dysfunction and lipodystrophies

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Congenital generalized lipodystrophy (CGL) is a rare, heterogeneous, autosomal recessive disorder characterized by the near-total absence of body fat with increased muscularity noticed at birth or in early infancy. Four distinct genetic subtypes of CGL have been reported to date. Types 1 and 2 are caused by biallelic variants in the 1-acylglycerol-3-phosphate-O-acyltransferase 2 (*AGPAT2*) and Berardinelli-Seip congenital lipodystrophy 2 (*BSCL2*) genes, respectively and are the most common subtypes (1). Types 3 and 4 are extremely rare and are caused by biallelic variants in the caveolin 1 (*CAV1*) (2), and caveolae-associated protein-1 (*CAVIN1*; also known as polymerase I and transcript release factor (*PTRF*)) genes (3), respectively. Patients with all CGL subtypes are predisposed to metabolic complications of insulin resistance, such as diabetes mellitus, hypertriglyceridemia, and hepatic steatosis; however, each subtype presents with some unique clinical features.

Type 3 CGL was initially reported in the landmark study of a single 20-year-old Brazilian female belonging to a consanguineous family with a homozygous p.(Glu38*) *CAV1* variant (2). She had generalized lipodystrophy, short stature, functional megaesophagus, severe hypertriglyceridemia, primary amenorrhea, chronic diarrhea, hepatic steatosis, and splenomegaly, and hypocalcemia presumed to be secondary to vitamin D resistance (2). She also had early onset acanthosis nigricans and hirsutism and developed diabetes at 13 years of age. She had absence of metabolically active adipose tissue, but mechanical adipose tissue and bone marrow fat were well-preserved (2). However, it was not clear which phenotypic features of this patient could be specifically attributed to the homozygous *CAV1* variant; additional patients were required to prove the disease-gene association.

A recent article published in this journal by Karhan *et al.* (4) has provided further compelling evidence on the association of CGL3 phenotype with a novel homozygous *CAV1* p.(His79Glnfs*3) variant by reporting the detailed phenotype of four additional patients, three females (age range, 8 months to 15 years) and one male (age 18 years), from a large Turkish kindred. All patients developed lipodystrophy in early infancy, had short stature, and failure to thrive, despite a voracious appetite. All three females had severe metabolic abnormalities including hypertriglyceridemia, low levels of high-density lipoprotein (HDL) cholesterol, and fasting hyperinsulinemia at a young age. The 15-year-old female had acanthosis nigricans, hirsutism, polycystic ovaries, and hepatic steatosis. The 18-year-old male had normal serum triglycerides and mildly decreased HDL-cholesterol and insulin resistance. Both older patients, but not the 10-year-old affected female, had dysphagia and megaesophagus due to achalasia requiring peroral endoscopic myotomy. Kim *et al.* (2) also reported functional megaesophagus, which was corrected at 15 years of age in their patient; however, further details were not provided. Karhan *et al.* (4) have suggested dysregulation of calcium homeostasis and excitation-contraction coupling in smooth muscle due to caveolae deficiency as potential cause of achalasia.

Kim *et al.* (2) had described hypocalcemia and renal calcium wasting, along with radiographic findings suggestive of osteopenia in their patient, requiring vitamin D supplementation. However, all four patients reported by Karhan *et al.* (4) had normal serum and urinary calcium levels. The two older patients, a 15-year-old female and 18-year-old male, had reduced bone mineral density suggestive of osteopenia (lumbar z score −2.78 and −3.85, respectively). Karhan *et al.* (4) also reported a bilateral loss of ellipsoid line and atrophy of the retinal pigment

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epithelium, suggestive of atypical retinitis pigmentosa in the 18-year-old male. Some recent studies have shown that *CAV1* is expressed in several retinal cell types and can modulate neuroprotective signaling (5). Interestingly, bilateral retinitis pigmentosa has been previously reported in two patients with a heterozygous *CAV1* p.(Lys135Argfs*4) variant, along with partial lipodystrophy and hypertriglyceridemia (6).

Karhan *et al.* (4) also reported six siblings with normal genotype and two siblings and seven parents with heterozygous *CAV1* p.(His79Glnfs*3) variant in the index kindred. They reported no lipodystrophy or metabolic derangements in the heterozygous carriers, although detailed anthropometric, metabolic, and bone density data were not available. For example, comparing the height and bone density of the affected homozygotes, heterozygous parents and siblings, and siblings with normal genotype would have confirmed their observation of short stature and osteopenia in the affected subjects. Furthermore, a whole exome or genome study of the family would have excluded other causal variants and their association, if any, with the phenotypes such as atypical retinitis pigmentosa.

Another intriguing fact is that while the heterozygous parents and siblings from the two CGL3 pedigrees harboring the null *CAV1* variants (i.e. p.(Glu38*) and p.(His79Glnfs*3)) have a normal phenotype consistent with autosomal recessive inheritance, previously other heterozygous variants c.-88delC, p.(Lys135Argfs*4), p.(Gln142*), p.(Phe160*), p.(Pro158Hisfs*23), and p.(Leu159Serfs*22) have been reported in association with pulmonary hypertension, and progressive right ventricular failure (7), partial lipodystrophy (6), and neonatal-onset lipodystrophy and progeroid syndrome (8). How these different heterozygous *CAV1* variants, even though they are all apparently null, cause phenotypes ranging from normal to severe syndromes is intriguing. There is a possibility that some of the heterozygous variants lead to a phenotype due to dominant-negative mechanisms, whereas the p.(Glu38*) and p.(His79Glnfs*3) variants do not because of true haploinsufficiency. The phenotypic variability among some of the heterozygotes could also be due to digenic interactions or polygenic influences.

The molecular basis of CGL3 and CGL4 implicates caveolar-dependent mechanisms in lipodystrophy. Caveolae are flask-shaped invaginations on the plasma membranes that maintain the integrity and function of the lipid droplets. They are mainly composed of lipids such as sphingolipids and cholesterol and proteins, such as caveolins and CAVINs. In addition, pacsin/syndapin

proteins, Eps15 homology domain proteins 1, 2, and 4, and receptor tyrosine kinase-like orphan receptor 1 (ROR1) have been recently reported to play a role in caveolae formation (9). *CAV1* and *CAV2* are expressed ubiquitously except in striated muscle and cardiac muscle cells, and *CAV1* is highly expressed in the adipocytes, endothelial cells, and fibroblasts; however, *CAV3* is muscle-specific (9).

Most interesting among these caveolar proteins is CAVIN1, as biallelic variants in this protein cause CGL4, which has many similar features to CGL3 (see Table 1). CAVIN1 anchors and stabilizes *CAV1* to the cytoskeleton in adipocytes, and CAVIN1 deficiency causes reductions of caveolae with a barely detectable expression of *CAV1* and *CAV2* and reduced expression of *CAV3* in human skeletal muscle (3). Similarly, skin fibroblasts of the patients with homozygous *CAV1* p.(Glu38*) and p.(His79Glnfs*3) variants did not exhibit any detectable protein expression of *CAV1* and strongly decreased expression of *CAV2* and CAVIN1 (4). Therefore, the observed overlapping phenotypic features between CGL3 and CGL4 are not surprising. In fact, unique clinical features of CGL3 and CGL4 can be exploited to further our understanding of the roles of *CAV1* and CAVIN1 in caveolae formation in various human tissues.

Patients with CGL4 have congenital pyloric stenosis; however, one patient has been described to have achalasia, diagnosed after she presented with dysphagia at 14 years of age (10). The autopsy report of a 14-year-old male with CGL4 showed thickened muscularis mucosa in the esophagus and gastro-duodenal junction, and prominent/large nerves in the esophagus, consistent with clinical features of esophageal dysmotility, infantile hypertrophic pyloric stenosis, and ileus in the patient (11). Pathophysiology of achalasia has been attributed to the functional loss of myenteric plexus ganglion cells in the distal esophagus and lower esophageal sphincter; however, the pathophysiology in CGL3 and CGL4 is unclear. Patients with type 4 CGL have congenital myopathy with high serum creatine kinase levels, 'muscle mounding', osteopenia, joint stiffness, atlantoaxial instability, and serious arrhythmias such as catecholaminergic polymorphic ventricular tachycardia, and sudden death (1). Similar to CGL3, metabolically active adipose tissue is absent in patients with CGL4, but mechanical adipose tissue and bone marrow fat are preserved. Table 1 describes unique clinical features for the CGL 3 and 4 subtypes.

Interestingly, knockout mouse models of *Cav1* and *Cavin1* also have distinct phenotypes. *Cav1*^{-/-} mice have

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Table 1 Clinical features of congenital generalized lipodystrophy type 3 and type 4.

	Type 3 CGL	Type 4 CGL
Growth	Short stature Failure to thrive in infancy Generalized lipodystrophy	Generalized lipodystrophy
Ophthalmologic	Retinitis pigmentosa*	None
Cardiovascular	None	Catecholaminergic polymorphic ventricular tachycardia Prolonged QT interval Sudden death
Gastrointestinal	Achalasia/megaesophagus Hepatic steatosis	Congenital pyloric stenosis Hepatic steatosis Achalasia*
Musculoskeletal	Osteopenia	Myopathy Muscle mounding Joint stiffness Atlanto-axial instability Osteopenia
Genitourinary	Polycystic ovaries* Amenorrhea	Azoospermia#
Neuro-cognitive	None	None

*Described in a single patient; #Described in autopsy (11).

hyperphagia but resistance to high-fat diet-induced obesity and have severely elevated post-prandial triglycerides and free-fatty acid levels; however, serum insulin, glucose, and cholesterol levels are entirely normal (12). They have a systemic decompensation in lipid accumulation with increasing age, resulting in dramatically smaller fat pads, reduced adipocyte cell diameter, and a poorly differentiated/hypercellular white adipose parenchyma. *Cavin1*^{-/-} mice, on the other hand, have high circulating triglyceride levels, reduced adipose tissue mass, hyperinsulinemia, and glucose intolerance, consistent with lipodystrophic phenotype. They lack morphologically detectable caveolae, have impaired triacylglycerol uptake and storage by adipocytes, and have hypertrophy of cardiomyocytes accompanied by progressive interstitial/perivascular fibrosis (3, 13). The relationship of these mouse models to the corresponding human deficiencies should be followed up.

In conclusion, the report by Karhan *et al.* (4) expands and provides further clarification of the CGL3 phenotype associated with homozygous *CAV1* null variants. The findings solidify the central role of caveolae in human lipodystrophy syndromes and are hypotheses-generating with respect to explaining the underlying phenotypic heterogeneity. Additional reports of this rare disorder in the future will be required to understand its natural history.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this commentary.

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