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Whole Exome Sequencing Identifies De Novo Heterozygous *CAV1* Mutations Associated with a Novel Neonatal Onset Lipodystrophy Syndrome

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Abstract

Despite remarkable progress in identifying causal genes for many types of genetic lipodystrophies in the last decade, the molecular basis of many extremely rare lipodystrophy patients with distinctive phenotypes remains unclear. We conducted whole exome sequencing of the parents and probands from six pedigrees with neonatal onset of generalized loss of subcutaneous fat with additional distinctive phenotypic features and report de novo heterozygous null mutations, c.424C>T (p. Q142*) and c.479_480delTT (p.F160*), in *CAV1* in a 7-year-old male and a 3-year-old female of European origin, respectively. Both the patients had generalized fat loss, thin mottled skin and progeroid features at birth. The male patient had cataracts requiring extraction at age 30 months and the female patient had pulmonary arterial hypertension. Dermal fibroblasts of the female patient revealed negligible *CAV1* immunofluorescence staining compared to control but there were no differences in the number and morphology of caveolae upon electron microscopy examination. Based upon the similarities in the clinical features of these two patients, previous reports of *CAV1* mutations in patients with lipodystrophies and pulmonary hypertension, and similar features seen in *CAV1* null mice, we conclude that these variants are the most likely cause of one subtype of neonatal onset generalized lipodystrophy syndrome.

Keywords

lipodystrophy; progeroid syndrome; caveolin 1; cataract; pulmonary hypertension

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher's web-site.

Conflict of interest: none.

INTRODUCTION

Genetic lipodystrophies are rare disorders characterized by body fat loss and predisposition to metabolic complications of insulin resistance [Garg, 2011]. In the last decade or so, mutations in several genes have been implicated in causing various subtypes of lipodystrophies [Garg, 2011]. For example, mutations in *AGPAT2*, *BSCL2*, *CAVI*, and *PTRF* are associated with congenital generalized lipodystrophies; mutations in *LMNA*, *PPARG*, *AKT2*, *PLIN1*, and *CIDEA* with familial partial lipodystrophies and mutations in *LMNA* and *ZMPSTE24* are reported in patients with Hutchinson–Gilford progeria and mandibuloacral dysplasia associated lipodystrophies [Garg, 2013]. Recently, mutations in *PSMB8*, *PCYT1A*, *PIK3R1*, *FOS*, and *POLD1* have been reported in patients with extremely rare subtypes of lipodystrophies [Agarwal et al., 2010; Dymant et al., 2013; Knebel et al., 2013; Thauvin-Robinet et al., 2013; Weedon et al., 2013; Payne et al., 2014]. Despite this progress, molecular basis of many extremely rare lipodystrophy patients with distinctive phenotypes remains unclear. Using whole exome sequencing in the parents and probands (trios), we report heterozygous de novo disease-causing mutations in *caveolin 1* (*CAVI*) gene in two patients presenting with neonatal onset of generalized loss of subcutaneous fat but sparing the buttocks with additional distinctive phenotypic features.

PATIENTS AND METHODS

All patients and their relatives provided written informed consent to participate in the study. For minors, their parents provided the consent. The study protocol was approved by the Institutional Review Board of UT Southwestern Medical Center. DNA was extracted from peripheral blood leucocytes from the patients and their relatives. Of the total of 14 probands referred to us with a novel subtype of generalized lipodystrophy presenting in the neonatal period, DNA from the parents and the patient (Trio) was available from six of them. Exome sequencing of five of these trios was performed at the University of Washington's Center for Mendelian Genomics (UW-CMG) and of one trio by Ambry Genetics.

Two of these patients, NLD 1500.4 and NLD 2800.5 were found to have heterozygous de novo novel loss of function mutations in the *CAVI* gene. Their clinical features are reported in detail here while the clinical features of the other four patients are summarized in Table I.

Patient 1 (NLD 1500.4)

This 7-year-old Caucasian boy from Spain underwent a detailed evaluation at UT Southwestern Medical Center at the age of 26 months (Fig. 1A and B, Table I). He was born full term at 39.5 weeks gestation with a birth weight of 2,930 g and a birth length 51 cm. He was noted to have thin skin, dysmorphic facies, and poor fat distribution (Fig. 2A and B). His feet and hands were bluish–purple. Although his linear growth had been normal, he had poor weight gain. He had no cognitive delay and had normal developmental milestones and teething. His hair was thin and grew slowly but the nails grew normally. He had frequent bowel movements, 4–5 times a day and increased thirst and polyuria which have spontaneously resolved at age 7. There was no parental consanguinity. He has two normal siblings.

Examination revealed generalized loss of subcutaneous fat, thin, dry skin with clearly visible dermal vessels suggestive of cutis marmorata (Fig. 1A and B). Subcutaneous fat was markedly deficient all over except for on the buttocks. He also had poor musculature. He had sparse blonde hair on the scalp, eyelashes, and eyebrows. The face was triangular with thin pointed nose and small mouth. The eyes were normal. He had no joint contractures. Cardiovascular and pulmonary examinations were normal. Abdomen was slightly protuberant and liver was just palpable under the costal margin. He had a normal phallus and the testes were descended. The fontanelles were closed. He had low set ears but had normal hearing. The fundus examination was normal.

Cardiac echocardiography and abdominal sonography were normal. Skeletal roentgenograms were normal with no evidence of clavicular or phalangeal resorption. Urinary osmolality was 726 mOsm/kg (normal range: 50–1,200 mOsm/kg) while his serum osmolality was 279 mOsm/kg (normal range: 278–305 mOsm/kg). His serum glucose, lipids, insulin, alanine aminotransferase, and aspartate aminotransferase levels were normal but alkaline phosphatase (742 IU/L; normal range: 50–300 IU/L) and lactate dehydrogenase (466 IU/L; normal range: 100–450 IU/L) levels were increased and HDL-cholesterol level was low (Table I).

At the age of 30 months, he was noted to have advanced bilateral cataract formation requiring surgery. Currently, he has sunken cheeks, pinched nose, and excessive wrinkling of the face and marked, generalized loss of subcutaneous fat except from the abdomen and buttocks. A recent echocardiography was normal with pulmonary artery pressure in the normal range. He had normal serum calcium, phosphate, alkaline phosphatase, and intact parathyroid hormone concentrations.

Patient 2 (NLD 2800.5)

This 22-month-old Caucasian girl was born at full term (Fig. 1C and D). Ultrasound examination in utero at 20 weeks gestation identified unilateral pleural effusion which resolved by 33 weeks gestation. She was small for gestational age and had pleural effusion at birth. She had a marked generalized lack of subcutaneous fat and mottled skin (Fig. 2C and D). She had a large fontanelle. She also had bilateral spontaneous pneumothorax which resolved after 4 days. She fed poorly and had dysphagia and chronic aspiration. At 2 months of age, a nasogastric tube was placed followed by a G-tube at age of 9 months. She has no cognitive delay. She had cyclical vomiting and diarrhea. The parents had no history of consanguinity. She had two older healthy brothers. She was taking lansoprazole and cyproheptadine.

Examination revealed generalized loss of subcutaneous fat, thin mottled skin with cutis marmorata (Fig. 1C and D, Table I). She had a triangular face with small lips. The anterior fontanelle was open. She had normal hearing. Teeth were small and yellowish. Eyes were normal with normal eyelashes and eyebrows. She had biparietal thinning of the scalp hair. She had no scoliosis or other joint problems. She had normal subcutaneous fat on the gluteal region. Her height was 81 cm with an arm span of 82 cm, arm length of 31 cm, and leg length of 38 cm. There was no asymmetry of the limbs or clinodactyly.

Her serum glucose, lipids, calcium, phosphorus, magnesium and vitamin D, prolactin, and insulin values were all normal but HDL cholesterol level was low (Table I). Computerized tomography of the chest at 18 months of age revealed pulmonary arteriovenous malformation in the left lower lobe. Lung biopsy revealed evidence of pulmonary arterial hypertension. Echocardiography at 28 months of age estimated pulmonary arterial pressure to be 60 mm Hg. Heart catheterization revealed pulmonary artery pressure of 40–50 mm Hg while right atrial pressure was 11 mm Hg. She is currently 36 months old. Molecular diagnostic studies for Hutchinson–Gilford progeria syndrome including genotyping for *LMNA* and *ZMPSTE24* were negative and microarray chromosomal analysis (1.8M probes by Quest) was normal.

Exome Sequencing

One out of six trios was processed by Ambry Genetics (~30 × coverage of the Agilent Sure SelectXT Human All Exon 50 Mb target, 2 × 100 bp Illumina HiSeq sequencing) while the other five trios were processed by the University of Washington Center for Mendelian Genomics (UW–CMG). The initial QC at UW–CMG entails DNA quantification, sex typing, and molecular “fingerprinting” using a 96-plex genotyping assay. Library construction (using 1 µg of genomic DNA) and exome capture are automated (Perkin–Elmer Janus II) in 96-well plate format. Libraries undergo exome capture using the ~63 Mb target from Roche/Nimblegen SeqCap EZ v3.0. Prior to sequencing, library concentration is determined by triplicate qPCR and molecular weight distributions are verified on Agilent Bioanalyzer 2,100. Individual exome multiplex-compatible libraries are pooled into sets of 24–96 samples using liquid handling robotics. Sequencing-by-synthesis is carried out on the Illumina HiSeq platform (2 × 50bp reads). Current throughput is sufficient to complete a 3–4 multiplexed exomes per lane at high coverage (60–80 × mean coverage).

Sequencing data processing involved: (i) base calling (Illumina RTA 1.13); (ii) demultiplexing and conversion into unaligned BAM files by lane and index sequence (Illumina Basecalls To Sam and Picard Extract Illumina Barcodes); (iii) alignment to the human reference (hg19/GRCh37) using BWA [Li and Durbin 2009]. Read-pairs not mapping within ± 2 standard deviations of the average library size (~125 ± 15 bp for exomes) were removed. Following QC, individual-level data were merged and subjected to the following steps: (i) base quality recalibration using GATK TableR-ecalibration; (ii) reads with duplicate start positions were removed; (iii) indel realignment using GATK Indel Realigner, and (iv) variant calling using multiple samples with the Unified Genotyper routine of GATK 1.6–11 [DePristo et al., 2011]. Multi-sample VCF files were filtered for 1,000 Genomes and NHLBI GO Exome Sequencing Project variants with a frequency of more than 1%, minimum reported quality values (QUAL ≥ 250, GQ ≥ 30, QD ≥ 5, AB ≥ 0.8), and minimum site coverage (DP) of 8. All remaining variants were required a mapability value of 1 (UCSC wg Encode-Duke Mapability-Uniqueness 35 bp). Variant effects were annotated using Ensembl Variant Effect Predictor [McLaren et al., 2010].

Sanger Sequencing

All three exons and the splice site junctions of *CAVI* were amplified with gene specific primers (available on request). Polymerase chain reaction (PCR) was assembled as described

earlier [Agarwal et al., 2002]. The PCR product was purified to remove primers and dNTP's and sequenced using Sanger technique with ABI Prism 3100.

Cell culture

Human dermal fibroblasts from one of the patients (NLD 2800.5) and a control subject were grown in Dulbecco's Modified Eagle Medium (DMEM) supplemented with antibiotic–antimycotic and 10% fetal bovine serum. Cell cultures were maintained in a humidified incubator at 37°C in 5% CO₂.

Immunofluorescence Staining

The cells were seeded on sterile glass 35 mm MaTTek dishes. Cells were washed with PBS, fixed with 3% paraformaldehyde in PBS containing 200 mM sucrose for 30 min and were permeabilized with 0.1% Triton X-100. After blocking the cells with blocking solution (10% goat serum plus 1% immunoglobulin free bovine serum albumin in phosphate buffered saline), they were incubated with the primary antibody, rabbit anti-Caveolin-1 (dilution 1:250, BD Transduction Lab) at 37°C for 1 h and visualized with the secondary antibody, goat-anti rabbit IgG-Alexa Fluor 568 (dilution 1:400, Life Technologies). Cell nuclei were stained with NucBlue (Life Technologies). Images were captured using the Leica SP5 Confocal microscope with 63× objective. All images were obtained at same laser power, gain, and offset. Quantitation was done using ImageJ. Cell numbers were counted with “Particle analysis” while total fluorescence was determined with “Thresholding”.

Transmission Electron Microscopy

The cells were fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, embedded in 2% agarose, post-fixed in buffered 1% osmium tetroxide containing 0.08% potassium ferricyanide, en bloc stained in 4% uranyl acetate, dehydrated with a graded series of ethanol, and embedded in EMbed-812 resin. Thin sections were cut on a Leica Ultracut UCT ultramicrotome and stained with 2% uranyl acetate and lead citrate. Images were acquired on a FEI Tecnai G2 Spirit electron microscope equipped with a LaB6 source and operating at 120 kV. Quantitation of plasma membrane cell surface and number of caveolae/cell was done using the TraxEM2 plugin in FIJI (ImageJ). Images from 10 different fibroblasts from control subject and 13 from the patient were stitched and the cell perimeter measured on the montaged cell. Caveolae were counted on the montages at a magnification of 100K.

RESULTS

Whole exome sequencing revealed a heterozygous de novo variant (chr7:116,199,228C>T) in *CAVI*, c.424C>T (p.Q142*), in NLD 1500.4, the proband of one of the trios sequenced at UW–CMG. Sanger sequencing confirmed the presence of this mutation in the proband, but not in the parents and an unaffected sibling of the patient (Fig. 3). All variants identified in this individual are described in Supplementary Table SI. Variants passed a semi-automated review process ruling out sequencing artifacts and excluding known polymorphisms above 1% allele frequency for recessive variants (including compound heterozygote and X-linked dominant) and 0.1% for de novo/dominant variants. They were also filtered for events

altering protein-coding sequence or potentially impacting transcript structure based on Ensembl Variant Effect Predictor annotation.

Exome sequencing of the NLD2, 800 trio was performed by Ambry Genetics. The bioinformatics analysis and filtering based on autosomal and X-linked dominant and autosomal recessive inheritance models revealed 23 genes (see Supplementary Table SII). Manual review to rule out sequencing artifacts and polymorphisms along with medical interpretation to rule out genes lacking clinical overlap with the patient's phenotype resulted in nine genes, which included eight "clinically novel" genes. Of these, one heterozygous variant (chr7:116,199,282–116,199,283 del) in *CAVI*, c.479_480delTT (p. F160*) was further investigated via co-segregation analysis. Neither the parents nor the two unaffected siblings had this variant, thus this was identified as a de novo variant in *CAVI*. Sanger sequencing further confirmed the presence of this mutation only in the proband, but not in the parents and unaffected siblings (Fig. 3).

These two patients did not share any de novo or other compound heterozygous alterations in other genes besides the *CAVI*. The other trios revealed shared variants in only *TTN* and *MUC16*, for which variants are commonly observed in exome sequencing experiments. Exome sequencing as well as Sanger sequencing of the other four patients did not reveal any disease-causing variants in *CAVI*. In addition, we sequenced *CAVI* by Sanger sequencing in eight additional patients with neonatal onset of lipodystrophy and progeroid features and did not find any disease-causing variants in any of them. Two patients had a heterozygous c.30+28C>T (rs35182456) variant and another one had a c.30 + 12_30 + 13insG (rs200963614) variant.

Immunofluorescence staining of the fibroblasts with antibody to the caveolin-1, revealed significantly reduced fluorescence in the patient's cells compared to the control subject's cells (Fig. 4). However, electron microscopy did not reveal any differences in the number of caveolae (mean 538 caveolae/mm in the control cells and 520 caveolae/mm in the patient's cells) or the morphology of caveolae (Fig. 4).

DISCUSSION

Both the patients shared many but not all clinical features. Both had enlarged triangular head with marked, generalized loss of subcutaneous fat with preservation of subcutaneous fat in the buttocks and the palms and soles. While their linear growth was normal, body weight gain was markedly lagging. Both of them had sparse scalp hair and had thin skin with visible blood vessels underneath (cutis marmorata). They did not have any metabolic abnormalities such as glucose intolerance or hypertriglyceridemia. There is resemblance of the phenotype to Wiedemann–Rautenstrauch Syndrome (WRS) [Wiedemann 1992; Rautenstrauch et al., 1994], also known as neonatal progeroid syndrome, but the definition of this entity has become significantly blurred due to the many misdiagnoses and needs careful re-evaluation before the exact relationship with our patients becomes clear. Although, the triangular face of patient 2 may have some overlap with Silver-Russell Syndrome [Wakeling 2011], there were no other features such as growth restriction, clinodactyly, body asymmetry, or shortening of the limbs.

In view of similarities in the phenotype of these two patients with neonatal onset of generalized lipodystrophy, previous association of *CAVI* heterozygous and homozygous variants with atypical partial lipodystrophy [Cao et al., 2008] and congenital generalized lipodystrophy (CGL), type 3 [Kim et al., 2008], respectively, and the low likelihood of chance occurrence of de novo *CAVI* variants in two out of six kindreds in which exome sequencing was performed ($P < 0.005$, assuming equal mutational burden and uniform gene likelihoods), we consider these *CAVI* variants to be most likely disease-causing.

Previously, *CAVI* mutations have been reported in patients with atypical partial lipodystrophy, congenital generalized lipodystrophy, type 3, and pulmonary hypertension [Cao et al., 2008; Kim et al., 2008; Austin et al., 2012] (Fig. 5) (Table II). Cao et al. [2008] reported heterozygous c.400delA, p.I134Lfs*5 mutation in a 55-year-old father and his 28-year-old daughter of Northern European origin with onset of lipodystrophy affecting the face and upper body at birth [Berger et al., 2002]. They had increased fat deposition in the gluteal region, thighs, calves, and visceral region. The daughter had extreme hypertriglyceridemia with recurrent attacks of acute pancreatitis while the father had diabetes and extreme hypertriglyceridemia. Besides these features they had neurologic abnormalities including severe ataxia and neuropathy. Both of them also had congenital cataracts and atypical retinitis pigmentosa [Berger et al., 2002]. Interestingly, one of our patients (NLD 1500.3) also developed cataracts at a very early age. Another patient, a 35-year-old male had a c.-88delC mutation in the 5' untranslated region and had loss of subcutaneous fat from the arms, gluteal region, thighs, and calves and increased visceral fat [Cao et al., 2008]. He had severe hypertriglyceridemia and recurrent episodes of acute pancreatitis. He had diabetes but no neurological deficits.

More recently, a heterozygous c.474delA (p.L159Sfs*22) variant in *CAVI* was found in a family with multiple subjects affected with pulmonary arterial hypertension [Austin et al., 2012]. Interestingly, several family members without detectable pulmonary arterial hypertension also carried the c.474delA *CAVI* mutation, suggesting incomplete penetrance. Another de novo *CAVI* variant c.473delC (p.P158Hfs*23) was found in a child with pulmonary arterial hypertension [Austin et al., 2012]. There was no mention of lipodystrophy among these patients. Interestingly, one of our patients (NLD 2800.5) has documented pulmonary artery hypertension both by echocardiography, cardiac catheterization and on lung biopsy.

Finally, Kim et al. [2008] reported a 20-year-old female from Brazil with CGL with short stature and primary amenorrhea, who had a homozygous p.E38* mutation in *CAVI*. As a young adult, this patient had near complete absence of the subcutaneous, intrathoracic, and intra-abdominal fat. She also developed insulin resistance, acanthosis nigricans, hirsutism, diabetes mellitus, and hypertriglyceridemia during childhood and had marked hypoleptinemia and hypoadiponectinemia. Other distinctive clinical features included hypocalcemia and hypomagnesemia, which were attributed to vitamin D resistance. At 15 years of age, surgical correction of functional megaesophagus was undertaken. Her echocardiogram was normal and she had no evidence of cardiomyopathy or pulmonary disease. Interestingly, the parents and two siblings who had heterozygous p.E38* mutation did not have any evidence of lipodystrophy.

CAVI is a major component of caveolae, which are specialized plasma membrane microdomains appearing as 50–100 nm vesicular invaginations [Stan 2005, Parton and Simons 2007], individually or in clusters, in adipocytes, endothelial cells, fibroblasts, and myocytes. *CAVI* is expressed ubiquitously but with high expression in the adipocytes, endothelial cells, and fibroblasts [Razani and Lisanti 2001]. *CAVI* encodes two isoforms of caveolin-1, α and β , using two alternate start sites [Scherer et al., 1995]. *CAVI* has a transmembrane domain, a Tryptophan-Tryptophan-domain for interaction with other proteins and is palmitoylated at the cysteine residues at 133, 143, and 156 position in the carboxy-terminus for cholesterol binding and interaction with other caveolar components [Dietzen et al., 1995]. Both its amino- and carboxy-termini are cytoplasmic. Caveolins play an important role in vesicular trafficking, homeostasis of cellular cholesterol, fatty acids and triglycerides, and signal transduction. The *Cav1*^{-/-} mice are viable and fertile but develop thick alveolar septa due to endothelium proliferation and fibrosis, pulmonary hypertension, right ventricular hypertrophy, cardiomyopathy, and increased susceptibility to tumorigenesis [Drab et al., 2001; Razani et al., 2001; Zhao et al., 2002; Le Lay and Kurzchalia 2005; Williams and Lisanti 2005]. Male *Cav1*^{-/-} mice have hypercalciuria and urinary bladder stones [Cao et al., 2003]. The *Cav1*^{-/-} mice also have reduced subcutaneous and intra-abdominal fat with underdeveloped perigonadal fat pads but not frank, generalized lipodystrophy [Razani et al., 2002]. Interestingly, brown adipose tissue is spared and undergoes hypertrophy [Razani et al., 2002]. These null mice also develop hypertriglyceridemia, hypoleptinemia, and hypoadiponectinemia [Razani et al., 2002]. The *Cav1*^{-/-} mice are resistant to weight gain upon feeding a high fat diet and develop postprandial hyper-insulinemia and insulin resistance [Cohen et al., 2003], suggestive of partial lipodystrophy.

Although we observed negligible expression of CAV1 in the skin fibroblasts of patient NLD 2800.5, we did not find any abnormality in the number or morphology of caveolae on electron microscopy. It is likely that caveolin-1 has minimal role in the formation of caveolae in the fibroblasts. Whether adipocytes could reveal abnormal caveolae morphology in our patients remains unclear due to lack of availability of adipose tissue.

This report extends the spectrum of disorders due to *CAVI* mutations to include one subtype of lipodystrophy. Our data further show that there are additional loci for neonatal onset lipodystrophy syndrome. How different mutations in *CAVI* cause such heterogeneous phenotypes such as CGL, acquired generalized lipodystrophy, cataracts, pulmonary hypertension and neonatal onset lipodystrophy, remains unclear.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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FIG. 1.

Physical features of our patients with neonatal onset lipodystrophy syndrome with *CAVI* mutations. Anterior (A) and posterior (B) views of patient 1 (NLD 1500.4) at 26 months of age, showing decreased subcutaneous fat except on the gluteal region and a triangular face with progeroid appearance. Anterior (C) and posterior (D) views of patient 2 (NLD 2800.5) at 22 months of age, showing decreased subcutaneous fat except at the gluteal region, a triangular face with progeroid appearance.

FIG. 2.

Physical features of our patients with neonatal onset lipodystrophy syndrome with *CAVI* mutations. Anterior (A) and lateral (B) views of the head of patient 1 (NLD 1500.4) at 4 months of age, showing decreased subcutaneous fat in the upper extremities, a triangular face with loss of hair and prominent sc blood vessels. Upper extremities also show prominent cutis marmorata. Anterior views of the head and upper body of patient 2 (NLD 2800.5) at 1 month (C) and 3 months (D) of age, showing marked loss of subcutaneous fat from the thorax and upper extremities, a triangular face with loss of hair and prominent sc blood vessels.

FIG. 3.

Pedigrees of the NLD patients and chromatograms showing the *CAVI* variants. A, B: NLD 1,500 and 2,800 Pedigrees, respectively. Affected individuals are shown as half-filled black symbols, unaffected subjects as unfilled symbols. Squares denote males and circles females. Slanted arrows indicate the probands. WT indicates the wild type sequence and the disease-causing heterozygous variants are reported in the probands. C: Normal chromatogram from Sanger sequencing of part of exon 3 of *CAVI*. The triplets encoding various amino acids are shown. D: Chromatogram of the corresponding region of exon 3 from the patient NLD 1500.4 showing the heterozygous c.424C > T variant resulting in substitution of the codon for amino acid glutamine at position 142 to a stop codon. E. Normal chromatogram of part of exon 3 of *CAVI*. E. Chromatogram of the corresponding region of exon 3 from patient NLD 2800.5 showing the heterozygous c.479_480delTT variant resulting in substitution of the codon for amino acid phenylalanine at position 160 to a stop codon.

FIG. 4.

Immunofluorescence CAV1 staining and morphology of caveolae of dermal fibroblasts of the NLD 2800.5 patient and control. A, B: Indirect immunofluorescence microscopy showing negligible fluorescence for caveolin-1 protein in the dermal fibroblasts from NLD 2800.5 patient compared to the fibroblast from a control subject. The staining in the NLD 2800.5 patient is significantly reduced. C: Quantitation of the fluorescence (red) intensity in the control and NLD 2800.5 cells. In all the experiments, cells were examined at the same laser power, gain and offset. In each field the total fluorescence intensity was divided by the number of nuclei (10–15) present to obtain fluorescence intensity/nucleus. Individual data points are shown as circles and median values as horizontal lines. Electron micrograph images of fibroblasts obtained from a control subject (D) and the NLD 2800.5 patient (E). Shown are the representative images with caveolae marked with arrowheads.

FIG. 5.

The gene structure of *CAVI* and the mutations reported in various patients. The boxes show the exons and the in between lines show the introns. Shaded areas within the boxes indicate the untranslated regions of exons 1 and 3. Arrows close to the exons represent the location of the primers used for sequencing. Mutations shown in bold font in exon 3 were found in our patients with neonatal onset of generalized lipodystrophy. Mutations above the gene structure are associated with lipodystrophy syndromes and below the structure with pulmonary hypertension.

TABLE I

Clinical features of the our patients with neonatal onset lipodystrophy syndrome

	NLD 1500.4	NLD 2800.5	NLD 1300.4	NLD 1600.3	NLD 2200.4	NLD 2300.4
Age at evaluation (y)	2.2	1.8	11.9	2.7	5.5	7.8
Current age (y)	7.0	3.0	17.8	?	10.4	11.9
Gender	M	F	F	M	F	F
Ethnicity	White	White	White	White	White	White
Hair	Thin	Thin	Thin	Thin	Thin	Normal
Gestational age (wk)	39.5	39.5	30	38.1	34	37
Birth Length (cm)	51	45.7	38	?	?	43
Birth occipito-frontal circumference (%ile)	10th	90th	3rd	>97th	?	?
Birth weight (g)	2,930	2,480	960	2,275	1,160	1,700
Current height, cm (%ile)	119 (25)	81 (90)	130 (<1)	65 (<1)	122 (1)	135 (10)
Current weight, kg (%ile)	18.5 (3)	7.9 (1)	28.6 (<1)	4.8 (<1)	14.6 (<1)	28.2 (6)
Occipito-frontal circumference, cm (%ile)	49.5 (10)	47 (60)	?	?	?	?
Mental development	Normal	Normal	Low	Normal	Normal	Normal
Anterior fontanelle open	–	+	+	+	+	–
Enlarged head	+	+	+	+	+	+
Poor weight gain	+	+	+	+	+	+
Chylothorax	–	+	?	?	?	?
Bilateral pneumothorax	–	+	?	?	?	?
Pulmonary hypertension		+	?	?	?	+ ^a
Arterio-venous malformation of chest	?	+	?	?	?	?
Cerebral atrophy	?	+	?	?	?	?
Triangular face	+	+	+	+	+	?
Polydipsia	+	?	–	?	?	–
Dry skin	+	?	+	?	?	+

	NLD 1500.4	NLD 2800.5	NLD 1300.4	NLD 1600.3	NLD 2200.4	NLD 2300.4
Mandibular hypoplasia	+	?	+	?	+	?
Lack of hair in posterior head	+	?	+	?	—	—
Prominent veins	+	+	+	+	+	—
Reduced subcutaneous fat	+	+	+	+	+	+
Thin nose	+	—	+	—	+	—
Small mouth	+	—	+	—	+	—
Liver enlargement	1 cm	—	—	?	—	?
White spots on teeth	+	?	?	?	?	?
Sparse lashes and eyebrows	+	—	+	+	—	—
Low set ears	+	—	+	—	—	+
Cataracts	+	—	—	—	—	—
Mottled skin	+	+	—	—	—	—
Sweating in head	?	+	+	?	—	—
SC fat on buttocks	+	+	+	?	+	+
Fat on palm and soles	+	+	?	?	Little	+
Other Features		Feeding tube at age 9 months	Constipation, loss of teeth, tube since 13 y, menarche age 13 y.	Multiple fractures at birth, finger contractures, club foot, undescended testes	hydrocephalous with ventriculo-peritoneal shunt, Entropion, on feeding tube, joint contractures	Strabismus, hyperopia, hydrocephalous with ventriculo-peritoneal shunt
Serum cholesterol (mg/dl) ^b	211	78	140	147	233	125
HDL-cholesterol (mg/dl) ^b	39	23	32	23	43	35
Triglycerides (mg/dl) ^b	131	52	285	266	173	124
Glucose (mg/dl) ^b	82	77	?	?	113	90
Insulin (μU/ml) ^b	3	2.2	?	?	?	?

+, yes; —, no; ?, not available; M, male; F, female; %ile, percentile.

^aSecondary pulmonary hypertension due to patent foramen ovale.

^bNormal values for serum cholesterol, 125–170 mg/dL.

HDL-cholesterol reference range not established; Triglycerides, 33–115 mg/dL; glucose, 65–99 mg/dL; insulin reference range not established.

TABLE II

Clinical features of patients with heterozygous or homozygous *CAV1* mutations

Austin et al. [2012] ^a							Cao et al. [2008]		
Patient ID	II-4	III-2	III-5	IV-1	IV-2	IPAH929	Patient A	Patient B	Patient C
CAV1 variant	c.474delA p. L159Sfs*22	c.474delA p. L159Sfs*22	c.474delA p. L159Sfs*22	c.474delA p. L159Sfs*22	c.474delA p. L159Sfs*22	c.4G3G>A p.V155I c.473delC p. P158Hfs*23	c.400delA p.H34Lfs*5	c.400delA p. H34Lfs*5	c.-88delC
Het/hom	het	het	het	het	het	het/het	het	het	het
Current age	71	46	48	25	21	5	28	55	35
Gender	M	F	F	M	F	F	F	M	M
BMI (kg·m ⁻²)	217	18.9	27.6	17	11	14.5	25	24.5	25.6
Pulmonary hypertension	+	+	+	+	+	+	?	?	?
Lipodystrophy	?	?	?	?	?	?	+	+	+
Diabetes	?	?	?	?	?	?	-	+	+
Hypertension	?	?	?	?	?	?	-	-	+
Hyperlipoproteinemia	?	?	?	?	?	?	+	+	+
Acanthosis nigricans	?	?	?	?	?	?	+	-	+
Congenital cataracts	?	?	?	?	?	?	+	-	+
Other features							nystagmus, spastic ataxia, dysdiadochokinesia, sensory neuropathy, retinitis pigmentosa	spastic ataxia, dysdiadochokinesia, sensory neuropathy, retinitis pigmentosa	recurrent pancreatitis

Patient ID	III-3	III-4	IV-2	IV-5	IV-4
CAV1 variant	c.112G>T p.Glu38*	c.112G>T p.Glu38*	c.112G>T p.Glu38*	c.112G>T p.Glu38*	c.112G>T p.Glu38*
Het/hom	het	het	het	het	horn
Current age	53	43	23	18	20
Gender	M	F	F	M	F
BMI (kg·m ⁻²)	?	22.3	28.3	38.8	20.9
Pulmonary hypertension	?	?	?	?	?
Lipodystrophy	-	-	-	-	+

Patient ID	Austin et al. [2012] ^a					Cao et al. [2008]			
	II-4	III-2	III-5	IV-1	IV-2	IPAH929	Patient A	Patient B	Patient C
Diabetes		−		+		−	−		+
Hypertension		+		+		−	−		?
Hyperlipoproteinemia		+		−		−	−		−
Acanthosis nigricans		?		?		?	?	+	+
Congenital cataracts		?		?		?	?		?
Other features	died of myocardial infarction						morbidly obese	hirsutism, primary amenorrhea, vitamin D resistance	

The *CAV1* variant c.4G3G>A [p.V155I] was predicted to be tolerated for protein function. Het, heterozygous; hom, homozygous; —, no; +, yes; ?, data not available; BMI, body mass index.

^aFour healthy subjects without detectable pulmonary arterial hypertension also harbored the c.474delA mutation, however, the details of these subjects were not reported suggesting incomplete penetrance.