A girl with CLOVES syndrome with a recurrent PIK3CA somatic mutation and pancreatic steatosis

Hiroaki Hanafusa1, Naoya Morisada1,2, Tadashi Nomura3, Daisuke Kobayashi4, Yoshinobu Akasaka5, Ming Juan Ye2, Kandai Nozu2, Noriyuki Nishimura2, Kazumoto Iijima2 and Hideto Nakao6

Abstract
CLOVES syndrome is characterized by congenital lipomatous overgrowth, vascular malformation, epidermal nevi, and scoliosis/spinal malformation. It is caused by somatic mosaicism of gain-of-function variants of PIK3CA. Here, we describe a novel case of a 5-year-old Japanese girl with CLOVES and concurrent pancreatic steatosis. She had a recurrent somatic mutation in PIK3CA (NM_006218.3: c.1357G>A, p.Glu453Lys), elevated HbA1c levels, and pancreatic steatosis. This case indicates that pancreatic screening is critical for PIK3CA-related disorders.

CLOVES syndrome (MIM #612918) is a rare disorder, and the abbreviation stands for congenital lipomatous overgrowth, vascular malformation, epidermal nevi, and scoliosis/spinal malformation1. CLOVES syndrome is generally caused by somatic mosaicism of gain-of-function variants in PIK3CA (3q26.32). In a previous report2, mutant allele frequencies were found to vary in the affected tissues, although the mutation rate in peripheral blood was very low. Therefore, it is important to analyze DNA derived from affected tissues and not from the peripheral blood for the diagnosis of CLOVES syndrome.

Pancreatic steatosis (PS), also called pancreatic lipomatosis, is frequently found in the adult pancreas and is typically benign. PS is identified by histological analysis or imaging, e.g., hyperechogenicity on abdominal ultrasonography and hypodensity of the pancreas on computed tomography3. PS is associated with obesity, increased age, and diabetes mellitus (DM)4. PS rarely occurs during childhood, and some genetic disorders have been associated with the development of PS5. However, to the best of our knowledge, PS has not been reported in patients with CLOVES syndrome. In this report, we describe a pediatric patient with CLOVES syndrome with concurrent PS.

The patient was a 5-year-old Japanese girl who was the second child of nonconsanguineous healthy parents. At 26 weeks of gestation, she was diagnosed with left pleural effusion and fetal hydrops by ultrasonography, and pleural drainage was performed. She was delivered by emergency cesarean section at 33 weeks of gestation because of increasing fetal hydrops. Her Apgar score was 5 (1 min)/7 (5 min), and her birth weight was 2604 g (> 97th percentile). She showed scattered capillary malformations in the upper and lower lips and both upper and lower limbs and enlargement of both second toes at birth. At 5 years, she was referred to our outpatient department. At that time, she showed systemic lipomatous overgrowth, particularly in the thorax and legs. Ultrasonography revealed lipomatous overgrowth and no development of mammary gland tissue. Scoliosis was observed by spinal X-ray examination (Fig. 1a). Enlargement of both second toes was still observed. Brain magnetic resonance imaging showed no abnormality, and she had no developmental...
disorders. Accordingly, we considered a diagnosis of CLOVES syndrome. Abdominal ultrasonography showed pancreatic hyperechogenicity (Fig. 1b), indicating PS. Her hemoglobin A1c (HbA1c) was elevated (6.1%). Her height at 5 years of age was 108.6 cm (−0.7SD), and her body weight was 21.9 kg (body mass index, 18.6).

To confirm her molecular diagnosis, we analyzed DNA samples derived from peripheral blood and affected adipose tissues using Sanger sequencing to detect the \textit{PIK3CA} variant after obtaining written informed consent from her parents. All procedures were reviewed and approved by the Institutional Review Board of the Kobe University School of Medicine and were performed in accordance with the ethical standards of the Declaration of Helsinki. We identified a heterozygous missense variant in \textit{PIK3CA} (NM_006218.3: c.1357G>A, p.Glu453Lys) in DNA derived from skin fibroblasts; no variant was observed in DNA derived from peripheral blood.

\textit{PIK3CA} encodes a 110 kDa catalytic subunit of phosphatidylinositol-3-kinase (PI3K). The PI3K/AKT/mammalian target of rapamycin pathway is involved in cell proliferation and cell growth. Somatic \textit{PIK3CA} variants cause abnormal activation of this pathway, and patients with \textit{PIK3CA} aberration show overgrowth syndromes (e.g., CLOVES syndrome and MCAP syndrome). The umbrella term “\textit{PIK3CA}-related overgrowth spectrum (PROS)” has been proposed. Mirzaa et al. hypothesized that phenotypic differences in overgrowth syndromes caused by somatic mosaic \textit{PIK3CA} variants may depend on the variant site and mosaic frequency. Piacci et al. reported a patient with different mosaic ratios of \textit{PIK3CA} p.Glu545Lys by tissue analysis and found no correlation between tissue-specific disease severity and mutant allele frequencies. We identified the mutant allele frequency of the patient to be 29.7% in affected tissue and 0.27% in white blood cells by ddPCR; hence, it is possible that mosaic frequency or differences...
in affected tissues might be involved. Further studies are required to explain these phenotypic differences.

The specific etiology of PS remains unclear. PIK3CA gain-of-function variants lead to overgrowth of fat tissues. Thus, patients with PROS are at risk of PS. Most patients with PS show a benign clinical course, but some may exhibit DM, pancreatic ductal failure, and pancreatic cancer. Our patient already showed elevated HbA1c levels, indicating that she was at high risk of DM and pancreatic cancer. Therefore, when clinicians encounter patients with PROS, pancreatic abnormalities should also be assessed.

In conclusion, our case demonstrated that pancreatic screening is essential for patients with PROS. Recently, specific therapy for PROS has been reported; thus, it is necessary to establish approaches for the definitive diagnosis of PROS.

HGV Database
The relevant data from this Data Report are hosted at the Human Genome Variation Database at https://doi.org/10.6084/m9.figshare.hgv.2585.

Acknowledgements
We thank the patient and her family. We thank Editage (www.editage.jp) for English language editing.

Author details
1Department of Clinical Genetics, Hyogo Prefectural Children’s Hospital, Kobe, Japan. 2Department of Pediatrics, Kobe University Graduate School of Medicine, Kobe, Japan. 3Department of Plastic Surgery, Kobe University Graduate School of Medicine, Kobe, Japan. 4Department of Orthopaedic Surgery, Hyogo Prefectural Children’s Hospital, Kobe, Japan. 5Department of Radiology, Hyogo Prefectural Children’s Hospital, Kobe, Japan. 6Department of Neonatology, Hyogo Prefectural Children’s Hospital, Kobe, Japan.

Conflict of interest
The authors declare that they have no conflict of interest.

Publisher’s note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Received: 16 February 2019 Revised: 3 June 2019 Accepted: 7 June 2019. Published online: 24 June 2019

References
1. Sapp, J. C. et al. Newly delineated syndrome of congenital lipomatous overgrowth, vascular malformations, and epidermal nevi (CLOVE syndrome) in seven patients. Am. J. Med. Genet. A 143A, 2944–2958 (2007).
2. Kurek, K. C. et al. Somatic mosaic activating mutations in PIK3CA cause CLOVES syndrome. Am. J. Hum. Genet. 90, 1108–1115 (2012).
3. Coulier, B. Pancreatic lipomatosis: an extensive pictorial review. J. Belg. Soc. Radiol. 100, 39 (2016).
4. Smits, M. M. & van Geenen, E. J. The clinical significance of pancreatic steatosis. Nat. Rev. Gastroenterol. Hepatol. 8, 169–177 (2011).
5. Mirzaa, G. et al. PIK3CA-associated developmental disorders exhibit distinct classes of mutations with variable expression and tissue distribution. JCI Insight 1, e87623 (2016).
6. Piacitelli, A. M. et al. Characterization of a severe case of PIK3CA-related overgrowth at autopsy by droplet digital polymerase chain reaction and report of PIK3CA sequencing in 22 patients. Am. J. Med. Genet. A 176, 2301–2308 (2018).
7. Keppler-Noreuil, K. M. et al. PIK3CA-related overgrowth spectrum (PROS): diagnostic and testing eligibility criteria, differential diagnosis, and evaluation. Am. J. Med. Genet. A 167A, 287–295 (2015).
8. Venot, Q. et al. Targeted therapy in patients with PIK3CA-related overgrowth syndrome. Nature 558, 540–546 (2018).