CLOVES syndrome caused by mosaic mutation in the PIK3CA gene identified in fibroblasts

Magdalena Klaniewska¹, Małgorzata Rydzanicz², Joanna Kosińska², Mateusz Biela¹, Anna Walczak², Elżbieta Szmid³, Anna Rozensztrauch¹, Rafał Płoski², Robert Śmigiel¹

¹Wroclaw Medical University, Poland
²Medical University of Warsaw, Poland

ABSTRACT

CLOVES syndrome is a rare dysmorphic syndrome with multiple defects caused by somatic activating mutations in the PIK3CA gene on chromosome 3q26.32. There are currently less than 200 individuals worldwide living with CLOVES syndrome (OMIM: 612918, ORPHA: 140944). Due to the extremely low prevalence rate of CLOVES syndrome, few epidemiological data are available in the literature.

We report a 4-year-old girl with somatic mutation in the PIK3CA gene (c.1357G>A) in fibroblast, revealed in the WES study, confirming the diagnosis of CLOVES syndrome.

CLOVES syndrome can be very difficult to diagnose, not only because of its extreme rarity, but also due to symptoms which vary both in range of symptoms and severity. Therefore, the case described by us may be helpful in the correct diagnosis of this rare disease in subsequent cases and makes an important contribution in rare disease diagnostics.

KEY WORDS:
mosaicism, CLOVES syndrome, PIK3CA mutation, overgrowth.

INTRODUCTION

For many years, overgrowth syndromes connected with vascular anomalies have been a challenge to diagnose. Recently, a separate group of patients has been identified with congenital lipomatous overgrowth, mixed vascular malformations, and dysregulated fat deposits [1]. The acronym CLOVE was first described by Sapp et al. in 2007 as congenital lipomatous (CL) overgrowth (O), vascular malformations (V), and epidermal nevi (E) [2]. Then Alomari in 2009 extended CLOVE to CLOVES to emphasize the association with scoliosis or skeletal and spinal anomalies and central nervous system malformations [3]. There are currently less than 200 individuals worldwide living with CLOVES syndrome (OMIM: 612918, ORPHA: 140944). The prevalence of the disease has been estimated at < 1/1,000,000.

We present a case report of young patient with CLOVES syndrome, and we point to the difficulties in diagnosis of a child with overgrowth and asymmetrical dysmorphic features including head, brain, face, subcutaneous and fat tissue, as well as skin.

CASE REPORT

A 4-year-old girl, born as in the 38th week of pregnancy via caesarean section because of macrosomia. The birth weight was 4300 g, length 59 cm, head circumference 42 cm, and chest circumference 34 cm. Apgar score was 9 points at the 1st minute. The pregnancy was uneventful. Family history was unencumbered.

Physical examination after birth revealed macrocephaly, facial dysmorphic features with asymmetry, and a congenital haemangioma. Ultrasound of the abdomen
and heart were normal. Cranial ultrasound scan and nuclear magnetic resonance, performed during the first days of life, revealed hemimegalencephaly, schizencephaly, and grey matter heterotopy (Fig. 1). In addition, gastroesophageal reflux disease (GERD) was diagnosed. The girl was initially suspected of Klippel-Trenaunay syndrome. Electroencephalography was abnormal but without any specific presentation. Infections from the TORCH group were excluded.

The girl was consulted by a clinical geneticist at the age of one year. Physical examination revealed low muscle tone, facial dysmorphic features with a leading sign of macrocephaly, and severe facial asymmetry including: eyelid fissures, cheeks, nose and mouth (Fig. 2). Extensive flat haemangiomas were observed mainly on the right side of the body. In addition, the child presented asymmetry of the lower and the upper limbs, especially asymmetry of the thigh and feet resulting from haemangiomas, as well as unidentified subcutaneous and fat tissue mass (Fig. 3). Other observed features were described: enlarged abdomen circumference (without liver and spleen enlargement), narrowed chest, and scoliosis. Moreover, macrodactyly of the third finger and a wide sandal gap were noticed. As a cause of these symptoms a mutation in the PIK3CA gene was suspected.

INVESTIGATIONS

Our patient firstly underwent targeted examination of the PIK3CA gene in chosen exons (2, 6, 8, 9, 20) by sanger sequencing from peripheral blood lymphocytes, which showed no pathogenic variants. For further examination, proband’s DNA purified from skin-derived fibroblast was subjected into whole exome sequencing (WES). A library was prepared using SureSelect Human All Exon v5 kit (Agilent, Santa Clara, CA, USA) and paired-end sequenced (2 × 100bp) on HiSeq 1500 (Illumina, San Diego, CA, USA). Bioinformatics analysis of raw WES data and variant prioritization were performed as previously described (PMID: 30254215). Selected variants were validated in proband’s fibroblasts and studied in proband’s blood as well as in blood collected from all available family members by amplicon deep sequencing performed using Nextera XT Kit (Illumina) and sequenced on a HiSeq 1500 (Illumina).
Considering the proband’s phenotype we prioritized a somatic missense heterozygous variant in the PIK3CA gene (hg19; chr3: g.178928079G>A, NM_006218.4:c.1357G>A, p.(Glu453Lys)) (Fig. 4A). The variant was absent in all tested databases, including our in-house database of > 1000 Polish exomes. In the ClinVar database p.(Glu453Lys) variant is described as probably pathogenic (https://www.ncbi.nlm.nih.gov/clinvar/variation/376470/). The presence of p.(Glu453Lys) was excluded in the proband’s parents (Fig. 4B).

**DISCUSSION**

CLOVES has been related to somatic activating mutations in the PIK3CA gene on chromosome 3q26.32 [4]. Somatic mutations appear during embryogenesis, resulting...
in two or more genetically separate cell lines. When
the mutation influences cell signalling pathways changes
in skin appearance can occur, occasionally with regional
hyperplasia or even tumour susceptibility. The phenotype
depends on the extent to which the mutation affects cel-


cular function as well as on the number and organization
of abnormal cells relative to normal cells [5]. PIK3CA is
an upstream regulator of the Akt-mTOR cell-signalling
pathway. This is balanced by the down-regulating activity
of tensin homologue protein and the phosphatase [1].

The key feature of the CLOVES syndrome is a trun-
cal lipomatous mass of variable size noted after birth [6].
Manifestations of overgrowth include asymmetric over-
growth with splaying of the feet and palmar or plantar
overgrowth. Skeletal anomalies in the form of progres-
sive scoliosis can occur [7]. Anomalies also affect feet and
hands. Most commonly they appear in the form of wide
feet and hands, macrodactyly typically involving the third
finger or third toe, and a wide sandal gap [8]. Abnormal-
ities seen occasionally include postaxial polydactyly and
pectus excavatum [7]. Low- and high-flow vascular mal-
formations belong to the major component of CLOVES
syndrome, as well. Venous malformations, in the form
of central, thoracic, and limb phlebectasia, have frequent-
ly been reported.

Central nervous system manifestations include hemi-
megalencephaly, polymicrogyria, a 4-layered cortex, ab-
normalities of the gray and white matter, ventriculomeg-
ally dysgenesis of the corpus callosum, neuronal migration
defects leading to seizures, tethered spinal cord, and neu-
ral tube defects [9]. Patients with CLOVES syndrome
have various degrees of intellectual disability. Almost all
patients with CLOVES syndrome are cognitively normal,
but one report by Alomari revealed that neurological
disorders were observed in about 50% of the study co-
hort [10]. CLOVES syndrome can be very tricky to di-
agnose, not only because of its extreme rarity, but also
because of symptoms that vary in both presentation and
severity.

The mutation indicated in our patient’s tests has been
described in the literature as associated with the PIK-
3CA-Related Overgrowth Spectrum (PROS). The term
was created in 2013 by the National Institute of Health
in Bethesda, to designate all the phenotypes caused by
PIK3CA mutation. The phenotypes that contain PROS
are as follows: megalencephaly, capillary malformation,
polymicrogyria syndrome (MCAP), congenital lipoma-
tous, overgrowth, vascular malformations, epidermal
nevi, scoliosis (CLOVES), dysplastic megalencephaly
(DMEG), fibroblast hyperplasia or overgrowth (FAO),
and macrodactyly [11]. These disorders are characterized
by a similar phenotype and overlapping of symptoms, so
it is often impossible to make a clear diagnosis.

With regard to diagnosis, CLOVES-specific mutations
are not well expressed in blood samples. Thus, genetic se-
quencing typically needs to require evaluation of at least

one tissue sample from affected and unaffected tissues. Chang et al. in 2017 explained that Sanger sequencing
may not be efficient for overgrowth syndromes char-
acterized by somatic variants in genes related to PI3K/
AKT/mammalian target of rapamycin (mTOR) [12]. In
the same research Chang et al. showed that next-genera-
tion sequencing (NGS) technology may help to identify
the low-level mosaicism and therefore enable diagnosis
of mosaic overgrowth syndromes [12].

Vascular malformations comprise a significant prob-
lem that affects patients with CLOVES syndrome. Venot et al. in 2018 reported that targeted treatment with
a PIK3CA inhibitor (BYL719) was free of drug-related
side effects in 19 patients with complex PROS vascular
malformations. The therapy revealed effective vascular
responses as well [13]. Currently BYL719 has entered
phase I-II clinical trials. Their purpose is to evalu-
ate BYL719 safety and efficacy in patients with various
PIK3CA-implanted solid tumours [11]. López Gutiér-
rez et al. in 2019 [14] reported a 17-year-old girl with
CLOVES syndrome, who had massive vascular malfor-
mations involving the external genitalia. She was taking
oral rapamycin, but with no effect. One month after start-
ing low-dose BYL719 treatment, the patient presented
a reduction in the size of the vascular malformations and
improved quality of life.

Gustin et al. in 2008 showed that somatic mutations
in the PIK3CA gene also occur at high frequency in
breast and other cancers [15]. Recent evidence suggests
that inhibitors of the phosphoinositidé 3 kinase (PI3K)
pathway, which are currently being tested in cancer, may
provide a treatment option for patients with overgrowth
syndromes [16]. Cancer therapeutic drug candidates
targeting the PI3K pathway offer hope of much-needed
targeted therapies for overgrowth syndromes. However,
trials require randomization, blinding, and placebo con-
trol to assess their true efficacy.

Taking into account the results of the molecular ex-
amination and the clinical picture, our patient was di-
agnosed with a PROS disorder. However, her symptoms
and somatic mutation in the PIK3CA gene (c.1357G> A)
revealed in the WES study strongly indicate the diagnosis
of CLOVES syndrome.

CLOVES syndrome is extremely rare. In summa-
ry, there is no absolute cure for CLOVES syndrome,
and all initiatives are targeted at improving the quality of life [12].

ACKNOWLEDGMENTS

We thank the patient and her family members for
their participation in this study.

This work was in part supported by a Statutory Grant
of Wroclaw Medical University (SUB.E160.19.004) as well
as by the National Science Centre (NCN) Poland grant
2017/25/N/NZ4/00250 to AW. DNA sequencing was

CLOVES syndrome caused by mosaic mutation in the PIK3CA gene identified in fibroblasts
carried out with the use of CePT infrastructure (Innovative economy 2007–13, Agreement POIG.02.02.00-14-024/08-00)

DISCLOSURE

The authors declare no conflict of interest.

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