Lymphangioma of the fetal neck within the PIK3CA-Related-Overgrowth Spectrum (PROS): A case report.

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Abstract

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Abstract

Neck tumors are rare fetal conditions. They can receive their growth stimuli by activating missense mutations characterizing disorders with benign overgrowth, collectively known as PIK3CA-Related-Overgrowth-Spectrum. This results in segmental overgrowth with phenotypic variation, genetic heterogeneity or tissue specific distribution. Thus, clinical and molecular diagnosis as well as treatment remains challenging.

Key Clinicals Message

The delineation of the prenatal diagnostic key features of PROS disorders will further assume a crucial part and a prenatal diagnosis of the causing mutations would provide physicians with a simplified interdisciplinary perinatal management.

Keywords: PIK3CA, PIK3CA-Related-Overgrowth-Spectrum, PROS, lymphangioma, lymphatic malformation, cervical, neck, fetal, prenatal, congenital.

Introduction

Congenital fetal neck tumors constitute exceedingly rare antenatal conditions. Prenatal diagnosis is usually straightforward by detailed sonographic examination. According to current literature these lesions are categorized into different types.[1–3] The most frequently described subtypes of neck tumors comprise lymphangiomas, followed by teratomas and hemangiomas.[1] However, the exact incidence of lymphangiomas remains unclear. It considerably varies whether pre- or postnatal cohorts are analyzed. Prenatal data suggest an estimated incidence of up to 1 in 1,000 live births.[2,4,5]

Although lymphangiomas are non-malignant vascular malformations of the lymphatic system and histologically mostly benign, extensive and heterogeneous tumor masses may compress vital cervical structures, so the final prognosis has been reported to be poor.[1,2,6,7]

The clinical course of lymphangiomas is related to the type of lymphatic malformation and varies from a mild symptomatic that tends to regress spontaneously to an aggressively invasive growth into surrounding vital structures.[5] Spontaneous regression in the latter cases is unlikely, but possible.[4,8] By compressing phenomena, polyhydramnios or airway obstruction can occur, a potentially life-threatening event.[5,6,8,9]

Lymphatic malformations like lymphangiomas usually arise from the defective embryological development of primordial lymphatic structures.[3,8,10] Recent data revealed that lymphangiomas receive their growth stimuli by activating missense mutations characterizing regional-located tumors in PIK3CA-Related Overgrowth Spectrum (PROS).[9,11–13]

PROS encompasses a group of disorders that are predominantly characterized by benign segmental overgrowth of several tissues with vascular and lymphatic malformations. Caused by heterozygous, mostly somatic mosaic-like pathogenic variants in the PIK3CA gene that arise post-zygotically, affected patients may present with regional-located or multiple-located findings.[6,9,14,15] The clinical picture strongly depends on the embryonic stage in which the causative mutation occurred, as well as the tissue type affected by this mutation.[15] Specific somatic activating mutations in the phosphatidylinositol-3-kinase/AKT/mTOR (PI3K-AKT-mTOR) pathway lead to heterogeneous segmental overgrowth phenotypes.[14]The PIK3CA gene encodes the 110 kD catalytic alpha subunit (p110α) of the PI3K protein complex, a lipid kinase of the PI3K-AKT-mTOR pathway, a signaling pathway, which is crucially involved in the regulation of cell proliferation, metabolism and survival as well as in angiogenesis. Hence, PI3K plays a key role in cell growth and division, cell migration and survival.[6,9,16] An altered activity of PI3K leads to uncontrolled cell division and as somatic mosaics, these mutations may trigger the development of overgrowth syndromes with segmental growth of several tissues with venous as well as lymphatic malformations to varying degrees.[6,9,13,16]

Over the past years, cancer associated PIK3CA mutations have been also reported to be responsible for a wide range of clinical benign overgrowth disorders.[6,17] Recent insights emphasize the topicality of PROS disorders.[9,13,18]

Lymphangiomas, developed regionally located in the context of PROS, are scarcely mentioned in the literature and informative case reports are almost completely missing. While the acronym PROS is an umbrella term for various clinical entities, even those entities itself, like lymphangiomas, can morphologically resemble as a chameleon.[14]

In the present report the prenatal and postnatal course of a fetus suffering from a huge cervical lymphangioma within the PROS will be delineated. We discuss the clinical picture to what is known from current literature

and focus on the targeted multidisciplinary approach to establish the final confirmation of an activating missense mutation-hotspot c.1633G>A;p.Glu545Lys identified by next generation sequencing.

Case presentation

Prenatal course and diagnostics

A 28-year-old III G I P gravida in the 28th week of pregnancy was referred to our prenatal diagnostic center due to the suspicion of a fetal neck tumor detected by prenatal ultrasound. The course of pregnancy was uneventful until the 28th week of pregnancy. The mother reported one prior spontaneous abortion and had no regular medication. The parents were of Caucasian origin and nonconsanguineous and the family history was otherwise unremarkable.

A detailed fetal anatomic ultrasound survey confirmed a fetal neck tumor with a dimension of 45 x 46 x 39 mm located at the anterior neck, presenting morphological features of a cystic-caverneous lymphhemangioma. The tumor extended from the right submandibular region across the front of the neck to the opposite side, with weak vascularization. The tumor was supposed to infiltrate the base of the mouth. Additionally, the mass could not be separated from the upper thoracic aperture (Fig. 1 a, b). With the exception of a polyhydramnios, no further sonomorphological abnormalitiy of the fetus was detected. The fetus showed an appropriate growth with an estimated fetal weight of 1,020 g corresponded to the 28th percentile. The parents decided against an amniocentesis. Subsequent ultrasound examinations within two to three months showed the growth of the tumor (73 x 68 x 41 mm), which was already strongly vascularized and now affecting the floor of mouth, but without identifying its entity beyond any doubt (Fig. 1 c, d). The parents were interdisciplinary counselled together with neonatologists and pediatric surgeons. Magnetic resonance imaging (MRI) was performed, confirming the inhomogeneity as well as the extent of the tumor but were also unable to clarify the definite tumor entity.

Postnatal neonatological intensive care, diagnostics, therapy and diagnosis of PROS

A female infant was delivered weighing 3,450 g (74thpercentile), 49 cm length (27th percentile) and 35 cm head circumference (71th percentile) by cesarean section with neonatological standby of an ex-utero intra-partum treatment (EXIT) procedure in 39th week of gestation (Fig. 2 a, b). The newborn adapted well and presented in good condition: APGAR 8/9/10, arterial umbilical cord pH 7.39. Initially, it required no active management and was immediately transferred to the neonatologist care unit. During inpatient stay, the infant underwent an extensive interdisciplinary neonatological intensive care with regular ultrasound and MRI follow-up (Fig. 2 c, d). The lymphangioma seemed to be stable. With intention of tumor regression as well as obtaining tissue, several sonographically controlled biopsies of the neck with multiple injections of Picibanil (OK-432) and the partial resection of areas of the tumor were performed. Attempting tumor size restriction with Propranolol, based on the histopathological suspicion of a hemangioma and the meanwhile significant tumor progression, failed. The histopathological findings and the clinical presentation of the tumor rather suggested a lymphangioma. In the meantime, an evaluation by ultrasound and MRIs of the neck showed a semicircular tumor extension, reaching cranially to the mastoid and intrathoracally to the carina with increasing infiltration of the base of the tongue and marked constriction of the cervical vessels without airway obstruction. With histopathological evidence of markers of the mTOR pathway, associated with a number of overgrowth disorders, a therapeutic approach with Sirolimus was initiated and resulted in a minimal size reduction. Nearly during the entire inpatient stay and despite an extensive interdisciplinary management, the etiology of the lympangioma remained unclear and the conventional molecular genetic investigation a challenging process. To establish a final diagnosis next generation sequencing (NGS) was performed and subsequently resulted in the detection of the missense mutation c.1633G>A;p.Glu545Lys.

Discussion

In certain cases of congenital fetal neck tumors, establishing a definite prenatal diagnosis might be challenging or even impossible. Despite all advances in imaging techniques available today it remains challenging to delineate the underlying cause of such etiologically heterogeneous tumors in utero. Both, the infant and

physicians, as well as the parents, will benefit from the most accurate clinical and, if possible, molecular pathological classification of the etiology of the tumor in order to be able to plan further interdisciplinary treatment. If the final diagnosis in this case presented had been known earlier, a number of therapeutic approaches could have been avoided.

During fetal life tumors of the neck tend to impair fetal swallowing, often resulting in polyhydramnios.[8] Another serious thread potentially arises from local compression phenomena.[6] Lymphatic malformations of the fetal neck with lymphangiomas constitute the most prevalent fetal neck mass.[1,2,4,8] In the last two decades there were a number of attempts to classify these lesions according to their etiological origin[8,10], morphological appearance[5,10], histological features[5,7] or postnatal course.[10]All these different classifications can coexist and their characteristics occur side by side. A common and generally accepted classification of lymphatic malformations is merely based on histological criteria. There are four distinct histological types of lymphangiomas consisting of capillary or cavernous lymphangioma, cystic hygroma and vasculolymphatic malformation.[7] However, Eivazi et al. stressed that this traditional histological classification is obsolete as with increasing frequency, it has been shown, that anterolateral lymphangiomas receive their growth stimuli by activating missense mutations characterizing regional-located tumors in PROS.[10–12] Axt-Fliedner et al. mentioned, that theories of origin of lymphatic malformations include the failure in connection between the lymphatic and the venous system and that they could be suitable to explain the origin of lymphangiomas embryologically.[5,8] However, they also emphasized, that in cases with late presenting, regional-located, anterolateral lymphangiomas, this theory has to be questioned.[8] It therefore might be that late-presenting lymphangiomas beyond 30 weeks of pregnancy rather arise as a result of direct alteration in DNA from a post-zygotic PIK3CA mutation.

Depending on time of post-zygotic cell division in embryogenesis, either somatic or, even rarer, constitutional mutations are the result.[6,9,15] As one of the most frequent hot-spot missense mutations, c.1633G>A;p.Glu545Lys was identified in the helical domain of PIK3CA.[6,16] Hot spot mutations, activating, gain-of-function variants, show significantly elevated biological and biochemical activities.[14,16] These are often associated with higher numbers of transformed cell foci suggesting more rapid cell proliferation.[17]

In 2015, diagnostic criteria have been defined by Keppler-Noreuil et al. to standardize entities, that originate from PIK3CA mutations, and to simplify the diagnostic work-up.[14] Accordingly, in the present case, the regional-located and sporadic overgrowth of predominantly lymphatic tissue of the fetal neck, already detected prenatally and classified as a lymphangioma, combined with the molecular biological detection of the somatic mutation in the PIK3CA gene with low-level mosaics, clearly point to the definitive diagnosis of a disorder within the spectrum of PROS.

Due to their inexorable growth throughout all tissue layers, diagnosis and treatment of lymphangiomas within PROS disorders is often difficult.[3,4,8] However, prenatal ultrasound remains the primary diagnostic method of choice in the detection of congenital tumors. Additional volume ultrasound, combined with color Doppler interrogation, may act as an adjunct in establishing the most likely diagnosis as it potentially provides valuable information regarding the spatial relationship to surrounding cervical structures and general extent of PROS disorders.[19] The value of an additional fetal MRI to accomplish the diagnostic work-up and to give further essential information, particularly in unfavorable maternal conditions (high Body-Mass-Index) and in complex fetal tumors with multiloculated masses in a variety of affected anatomical regions has been highlighted by a number of publications.[8,19,20] Combined with information from 3D volume ultrasound, it can increase the accuracy of prenatal diagnosis including location, architecture of tissue, volume or intracranial or thoracic spread.[18,19]

Histopathologically, in the present case, the cyst wall-coating cells presented themselves without expression of D2-40, a lymphatic endothelial marker, but showed clear CD31 expression, characterizing blood vascular endothelial cells, so that the finding was classified most likely as a cavernous hemangioma due to the complex histologic pattern (Fig. 3). But considering the clinical aspects, a lymphangioma should be assumed. The D2-40 negativity does not exclude a lymphangioma. A distinct histological or immunohistochemical differentiation between a lymphangioma and a hemangioma was not possible. Due to the histological expression

pattern ($CD31^+$/D2-40^-), it is most likely, that endothelial cells harbor the PIK3CA mutation.[13,21]

All case reports published so far highlight the complexity of prenatal diagnosis of somatic mosaic mutations.[9,18] In 2014, Keppler-Noreuil et al. also defined testing eligibility criteria for somatic PIK3CA mutations.[17] Adequate tissue sampling is already a challenge postnatally – and, above all, as a highly invasive procedure, prenatally.[17,18] Maybe cultured amniocytes obtained by amniocentesis for prenatal diagnosis might be promising and are less invasive.[9,17,18]

Using NGS, the sensitivity of genetic testing has improved significantly and molecular diagnosis of somatic overgrowth has become feasible.[15–17] By now, mosaic levels can be detected with a variant allele frequency (VAF) down to 1 %.[16]In the tissue received in this case from multiple biopsies, the activating missense mutation-hotspot c.1633G>A;p.Glu545Lys with low-grade somatic mosaic and a VAF of 6.5 % was detected. This is one of the most frequent mutation hotspots in the PIK3CA gene, causing somatic overgrowth in PROS. Only with the change from the pure morphologic description of lymphangiomas to the recognition of their molecular causes, they can be categorized into the spectrum of PROS diseases.

The identification of a component of the PI3K-AKT-mTOR signaling pathway can partly explain the effect of the mTOR-inhibitor Sirolimus, which resulted temporarily in a minimal size reduction. The targeted application of a PIK3-inhibitor could be more promising, even as a serious alternative treatment to surgical debulking. Finally, obtaining tissue for histopathological and molecular genetic examination to confirm the definitive diagnosis is mandatory.[18]

From a therapeutic perspective, debulking procedures were the most promising approaches yet.[3–6,8,13] However, in the future, a shift from surgical debulking to personalized, targeted pharmacological intervention has been proposed.[15,18]Pharmacological therapy approaches are sclerosing therapy of fetal neck lymphangiomas with Bleomycin or Picibanil (OK-432), maybe prenatal. In addition, various molecule inhibitors, targeting different components of the PI3K-AKT-mTOR signaling pathway, are under clinical investigation: mTOR-inhibitors like Sirolimus (SRL or Rapamycin) or Everolimus (RAD-001) and recently, PIK3-inhibitors like Alpelisib (BYL719). Due to the increasing understanding of molecular biology of PROS disorders, targeted therapies are highly promising and less invasive. Moreover, it can improve quality of life of affected patients.[2,4–6,10,13,22]

Despite all efforts, there is a high risk of recurrence.[4,10] Fetal prognosis of congenital tumors of the neck depends largely on the nature of lesion, on their location and size, the affected surrounding structures as well as the presence of other anomalies.[7,20]

Conclusion

PROS encompasses rare genetic disorders resulting from somatic, mosaic gain-of-function mutation of the PIK3CA gene. As a result of the genetic mosaicism, the clinical presentation is extremely variable. Comprehensive diagnostics are expensive, time-consuming and they place an unnecessary burden on the infant and its family. A prenatal diagnosis of this extremely rare mutation enables physicians a simplified interdisciplinary perinatal management. Moreover, it offers parents and family members to be involved in the assessment process in the context of an informed consent. Nevertheless, diagnosis of PROS remains challenging, both pre- and postnatally and unfortunately, the diagnosis is rarely made prenatally. Therefore, physicians and parents stay in the dark for a long time regarding a definitive diagnosis and promising therapy.

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References

1. Kamil D, Tepelmann J, Berg C, et al. Spectrum and outcome of prenatally diagnosed fetal tumors. *Ultrasound Obstet Gynecol* . 2008;31(3):296-302. doi:10.1002/uog.5260

2. Mikovic Z, Simic R, Egic A, et al. Intrauterine treatment of large fetal neck lymphangioma with OK-432. *Fetal Diagn Ther* . 2009;26(2):102-106. doi:10.1159/000238111

3. Amodeo I, Colnaghi M, Raffaeli G, et al. The use of sirolimus in the treatment of giant cystic lymphangioma: Four case reports and update of medical therapy. *Medicine (Baltimore)* . 2017;96(51):e8871. doi:10.1097/MD.0000000000008871

4. Knipping S, Bau V. Lymphatische Malformationen im Kopf-Hals-Bereich: Erfahrungen mit Sklerosierungstherapie. *HNO* . 2011;59(7):683-688. doi:10.1007/s00106-011-2284-1

5. Grasso D, Pelizzo G, Zocconi E, Schleef J. Lymphangiomas of the head and neck in children. *Acta Otorhinolaryngol Ital* . 2008;28(1):17-20.

6. Madsen RR, Vanhaesebroeck B, Semple RK. Cancer-Associated PIK3CA Mutations in Overgrowth Disorders. *Trends Mol Med* . 2018;24(10):856-870. doi:10.1016/j.molmed.2018.08.003

7. Cho JY, Lee YH. Fetal tumors: prenatal ultrasonographic findings and clinical characteristics. *Ultrasonography* . 2014;33(4):240-251. doi:10.14366/usg.14019

8. Axt-Fliedner R, Hendrik HJ, Schwaiger C, Ertan AK, Friedrich M, Schmidt W. Prenatal and perinatal aspects of a giant fetal cervicothoracal lymphangioma. *Fetal Diagn Ther* . 2002;17(1):3-7. doi:10.1159/000047996

9. De Graer C, Marangoni M, Romnée S, et al. Novel features of PIK3CA-Related Overgrowth Spectrum: Lesson from an aborted fetus presenting a de novo constitutional PIK3CA mutation. *Eur J Med Genet* . 2020;63(4):103775. doi:10.1016/j.ejmg.2019.103775

10. Eivazi B, Werner JA. Lymphatische Malformationen im Kopf-Hals-Bereich. *HNO* . 2014;62(1):6-11. doi:10.1007/s00106-013-2803-3

11. Luks VL, N K, Mp V, et al. Lymphatic and other vascular malformative/overgrowth disorders are caused by somatic mutations in PIK3CA. *J Pediatr* . 2015;166(4):1048-54.e1. doi:10.1016/j.jpeds.2014.12.069

12. Rodriguez-Laguna L, Agra N, Ibañez K, et al. Somatic activating mutations in PIK3CA cause generalized lymphatic anomaly. *J Exp Med* . 2019;216(2):407-418. doi:10.1084/jem.20181353

13. Le Cras TD, Goines J, Lakes N, et al. Constitutively active PIK3CA mutations are expressed by lymphatic and vascular endothelial cells in capillary lymphatic venous malformation. *Angiogenesis* . 2020;23(3):425-442. doi:10.1007/s10456-020-09722-0

14. Keppler-Noreuil KM, Rios JJ, Parker VER, et al. PIK3CA-Related Overgrowth Spectrum (PROS): Diagnostic and Testing Eligibility Criteria, Differential Diagnosis, and Evaluation. *Am J Med Genet A* . 2015;0(2):287-295. doi:10.1002/ajmg.a.36836

15. McNulty SN, Evenson MJ, Corliss MM, et al. Diagnostic Utility of Next-Generation Sequencing for Disorders of Somatic Mosaicism: A Five-Year Cumulative Cohort. *Am J Hum Genet* . 2019;105(4):734-746. doi:10.1016/j.ajhg.2019.09.002

16. Spier I, Aretz S. Überwuchssyndrome durch Mutationsmosaike im PI3K-AKT-Signalweg. *medgen* . 2017;29(3):306-313. doi:10.1007/s11825-017-0153-3

17. Keppler-Noreuil KM, Sapp JC, Lindhurst MJ, et al. Clinical delineation and natural history of the PIK3CA-related overgrowth spectrum. *Am J Med Genet A* . 2014;164A(7):1713-1733. doi:10.1002/ajmg.a.36552

18. Emrick LT, Murphy L, Shamshirsaz AA, et al. Prenatal diagnosis of CLOVES syndrome confirmed by detection of a mosaic PIK3CA mutation in cultured amniocytes. *Am J Med Genet A* . 2014;164A(10):2633-2637. doi:10.1002/ajmg.a.36672

19. Tseng J-J, Chou M-M, Chen W-H. Prenatal 3- and 4-dimensional Ultrasonographic Findings of Giant Fetal Nuchal Hemangioma.*Journal of the Chinese Medical Association* . 2007;70(10):460-463. doi:10.1016/S1726-4901(08)70040-6

20. Knox EM, Muamar B, Thompson PJ, Lander A, Chapman S, Kilby MD. The use of high resolution magnetic resonance imaging in the prenatal diagnosis of fetal nuchal tumors. *Ultrasound in Obstetrics & Gynecology* . 2005;26(6):672-675. doi:https://doi.org/10.1002/uog.2601

21. North PE. Classification and Pathology of Congenital and Perinatal Vascular Anomalies of the Head and Neck. *Otolaryngol Clin North Am* . 2018;51(1):1-39. doi:10.1016/j.otc.2017.09.020

22. Keppler-Noreuil KM, Parker VER, Darling TN, Martinez-Agosto JA. Somatic Overgrowth Disorders of the PI3K/AKT/mTOR Pathway & Therapeutic Strategies. *Am J Med Genet C Semin Med Genet* . 2016;172(4):402-421. doi:10.1002/ajmg.c.31531