

CASE REPORT

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A 16q deletion involving *FOXF1* enhancer is associated to pulmonary capillary hemangiomas

Patrizia Dello Russo¹, Alessandra Franzoni¹, Federica Baldan², Cinzia Puppini², Giovanna De Maglio¹, Carla Pittini³, Luigi Cattarossi³, Stefano Pizzolitto¹ and Giuseppe Damante^{1,2*}

Abstract

Background: Pulmonary capillary hemangiomas (PCH) is an uncommon pulmonary disorder, with variable clinical features depending on which lung structure is affected, and it is usually linked to pulmonary arterial hypertension. Congenital PCH has been very rarely described and, so far, the only causative gene identified is *EIF2AK4*, which encodes for a translation initiation factor. However, not all PCH cases might carry a mutation in this gene.

Case presentation: We report the clinical and cytogenetic characterization of a patient (male, newborn, first child of healthy non-consanguineous parents) died after three days of life with severe neonatal pulmonary hypertension, due to diffuse capillary hemangiomas diagnosed post mortem.

Conventional karyotyping, Microarray-Based Comparative Genomic Hybridization (CGHa) and quantitative PCR were performed. CGHa revealed a heterozygous chromosome 16q23.3q24.1 interstitial deletion, spanning about 2.6 Mb and involving a *FOXF1* gene enhancer. Quantitative PCR showed that the proband's deletion was *de novo*. Microsatellite analysis demonstrate that the deletion occurred in the maternal chromosome 16.

Conclusion: *FOXF1* loss of function mutation have been so far identified in alveolar capillary dysplasia with misalignment of pulmonary veins (ACD/MPV), a lung disease different from PCH. Our data suggest the hypothesis that disruption of the *FOXF1* gene enhancer could be a genetic determinant of PCH. Moreover, our findings support the idea that *FOXF1* is a paternally imprinted gene.

Keywords: Pulmonary capillary hemangiomas, Chromosomal abnormalities, Deletion, Gene regulation

Background

Pulmonary capillary hemangiomas (PCH) is a rare disorder that was first reported in 1978, with less than a few hundred nonrelated cases reported, so far [1]. The PCH frequency within the general population is actually unknown [2, 3]. PCH anatomopathological features are pulmonary hypertension and excessive neovascularization characterized by capillary-sized blood vessels within the pulmonary interstitial tissue, vasculature, and airways [4, 5]. Clinically, PCH cases are quite variable; also

because, this disease could mimic different lung diseases, including pulmonary veno-occlusive disease, idiopathic pulmonary arterial hypertension and atypical interstitial lung disease [2]. Nowadays, the only genetic cause identified is a mutation in *EIF2AK4* gene (*Eukaryotic translation initiation factor 2 alpha kinase 4, in chromosome 15q15.1*), which encodes for a translation factor [6]. However, not all PCH cases might carry a mutation in this gene [2].

We report the clinical and genetic characterization of a newborn male presenting early severe pulmonary hypertension, due to a post-mortem diagnosis of PCH.

He carried a 2.6 Mb sized 16q23.3q24.1 deletion, as demonstrated by CGHa. He displayed no additional anomalies or malformations, and no familial occurrence.

* Correspondence: giuseppe.damante@uniud.it

¹Dipartimento di Medicina di Laboratorio, Azienda Ospedaliero-Universitaria S. Maria della Misericordia, Udine, Italy

²Dipartimento di Scienze Mediche e Biologiche, Università di Udine, Piazzale Kolbe 4, 33100 Udine, Italy

Full list of author information is available at the end of the article

Case presentation

Clinical report

The patient was the first child of nonrelated healthy parents with a negligible familial history. The patient was a full term (41 weeks) male infant, born by induced vaginal delivery to a 38-year old mother, whose pregnancy was uneventful. Birth weight, length and head circumference were 3340 gr, 50.2 cm and 34.0 cm, respectively. Apgar scores were 4-10-10. After birth, since the patient did not show spontaneous breathing and was hypotonic, he was stimulated and ventilated by ambu (FiO₂: 0.4). Because he presented an increased O₂-requirement after one hour of life, he was subjected to oxygenation by FiO₂ 40 %. Chest radiography showed widespread hypopneumonia of the right hemithorax. Blood tests were negative for infection. Ultrasound revealed a normal brain anatomy, with mild hyperechoic periventricular left rear. Ultrasonography was compatible with wet lung disease. 21 h after birth, the infant developed a deteriorating respiratory distress and echocardiography documented a severe pulmonary hypertension in the absence of congenital structural abnormalities. He died at the third day of life from respiratory distress, pulmonary hypertension, heart failure and an extreme bradycardia. The autopsy demonstrated a lung parenchyma characterized by marked edema and stasis.

Materials and methods

Histology and immunohistochemistry

Tissue from autopsic lung samples were fixed in 4 % formalin for about 24 h. Fixed tissue was then paraffin embedded and 4 μm slides were stained with hematoxylin and eosin. Immunohistochemical stains for CD31 (1:200, clone JC70A, Dako, Denmark), CD34 (1:50, clone QBEND10, Dako, Denmark) and histochemical Masson's trichrome stain were performed for vascular pattern observation.

Cytogenetics and molecular genetics

Blood samples of proband and parents were obtained after a signed informed consent. Parents informed consent was also given to analyse their own genomes. We explained that analysis of the parents would be useful to better understand the proband results. When CGHa is performed, obtaining blood samples from parents is part of our standard care.

Conventional high resolution karyotyping (GTG banding) was performed on proband blood lymphocytes following conventional procedures.

For CGHa analysis genomic DNA was isolated from an EDTA peripheral blood sample using QIAamp Blood Midi Kit according to the manufacturer procedure (Qiagen, Hilden, Germany). Molecular karyotyping was performed in accordance with the manufacturer procedure with an

180,000-oligonucleotide microarray (Sure Print G3 Human CGH Microarray Kit 180 k, Agilent Technologies, Santa Clara, CA, USA). The genomic sample was labelled and hybridized according with the Agilent Enzymatic Labelling protocol. We used a male DNA Coriell GM10851 (Coriell Institute, Camden, NJ, USA) as a normal reference. CGH Agilent Genomic Workbench Lite Edition 6.5.0.18 software and UCSC hg19 assembly were used to analyze the results. The presence of a copy number variation was defined by the presence of an abnormal log₂ ratio for at least three contiguous oligonucleotides. The presence of the deletion in our patient was confirmed by quantitative PCR using 7300 Real Time PCR System (Applied Biosystems, Foster City, CA, USA) (data not shown). Primers inside *OSGIN1*, *EMC8*, *ZDHHC7* genes were employed.

Microsatellites located into the deletion were analyzed by PCR and capillary electrophoresis sizing of the products. Microsatellites were amplified using the following primers:

L17941, Forward: 5'-6FAM-CTGGGTA CTCTTC TGTGACA-3';

Reverse: 5'-CTCTCTCCCCAACATGGTG-3'.

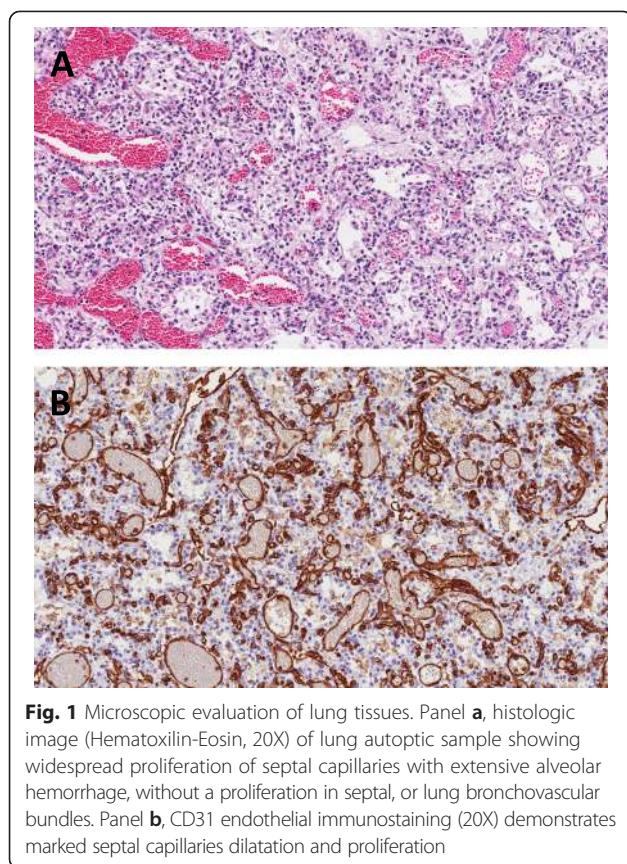
L29692, Forward: 5'-6FAM-TGTGTGTCTTCTG GGGGAGT-3';

Reverse: 5'-CACAGCTAGCCACAGGCAG-3'

The amplified products were analyzed by capillary electrophoresis (3500 IDX sequencer, Life Technologies).

Results

Histopathological analysis of the lungs showed marks of PCH (Fig. 1, panel a). Sections of the lung reveal lobular architecture with acinar underdevelopment and numerous dilated vascular channels within the thickened septa. Reduced radial-alveolar count was detected, reflective of decreased alveolarization. Air-blood barriers were detected within the alveolar walls; however, a rich, congested, proliferated capillary network was present within interalveolar septa. This is associated with some hemorrhage into the airspace as well as some regions with large number of histiocytes in the airspaces. Preacinar and intra-acinar arteries had thick smooth muscle medial coats and there was an extension of smooth muscle into the peripheral intra-acinar arteries up to the alveolar wall level. In addition, a thick collagen adventitial coat was present around the preacinar arteries. These structural features, are reminiscent of those present in persistent pulmonary hypertension of the newborn. Abnormalities of pulmonary lymphatics were not present. No pulmonary venous misalignment or obstructive/occlusive changes are discerned. Immunohistochemical analysis of CD31 demonstrates septal capillaries



dilatation and proliferation (Fig. 1, panel b). Altogether, these features indicate the presence of an intractable hypertensive pulmonary vascular disease of the newborn consistent with the morphologic characteristics of pulmonary hemangiomatosis with structural abnormalities in preacinar and intra-acinar pulmonary arteries (“post-resistant segment of the vasculature”).

Standard karyotyping showed no abnormalities (46,XY).

CGHa identified a submicroscopic deletion in 16q23.3q24.1 cytoband (deletion size 2.6 Mb) (Fig. 2). The deletion spans from 83,676,990 to 86,292,585 bp, encompassing LINC01082 and disrupting LINC01081, which are long non-coding RNAs located in the *FOXF1* tissue-specific distant enhancer, mapping 0.3 Mb upstream *FOX* genes cluster [7]. Positions are referred to Genome Assembly hg19. In the Additional file 1: Table S1 all genes contained in the deletion are listed. Among them, only the *DNAAF1* gene deficiency is known to cause a lung disease, i.e. the primary ciliary dyskinesia (or Kartagener syndrome), in which, however, airways cilia, but not alveoli and their vessels, are affected.

No other pathogenetic genomic imbalance was detected in the proband sample. Deletions in this region have been recently found in patients with Alveolar Capillary Dysplasia with Misalignment of Pulmonary Veins (ACD/MPV) [7]. In Fig. 3 are shown all deletions of the *FOXF1* region

so far identified in patients with ACD/MPV. PCH and ACD/MPV are two different diseases related to opposite phenomena in lung development disruption. In fact, PCH is characterized by capillaries proliferation in pulmonary interstitium [4, 5], while in ACD/MPV immature lobular development and reduced capillary density are present [8, 9]. In our patient, acinar underdevelopment and decreased alveolarization were present, which are reminiscent of ACD/MPV. However, we did not find the other typical feature of ACD/MPV, i.e. misalignment of pulmonary veins, in our case. Instead, the major histological finding of our patient was the capillary proliferation within the interalveolar septa, which is typical in PCH.

By quantitative PCR, our patient parents were evaluated: both subjects presented no abnormalities, thus suggesting the *de novo* origin of the deletion.

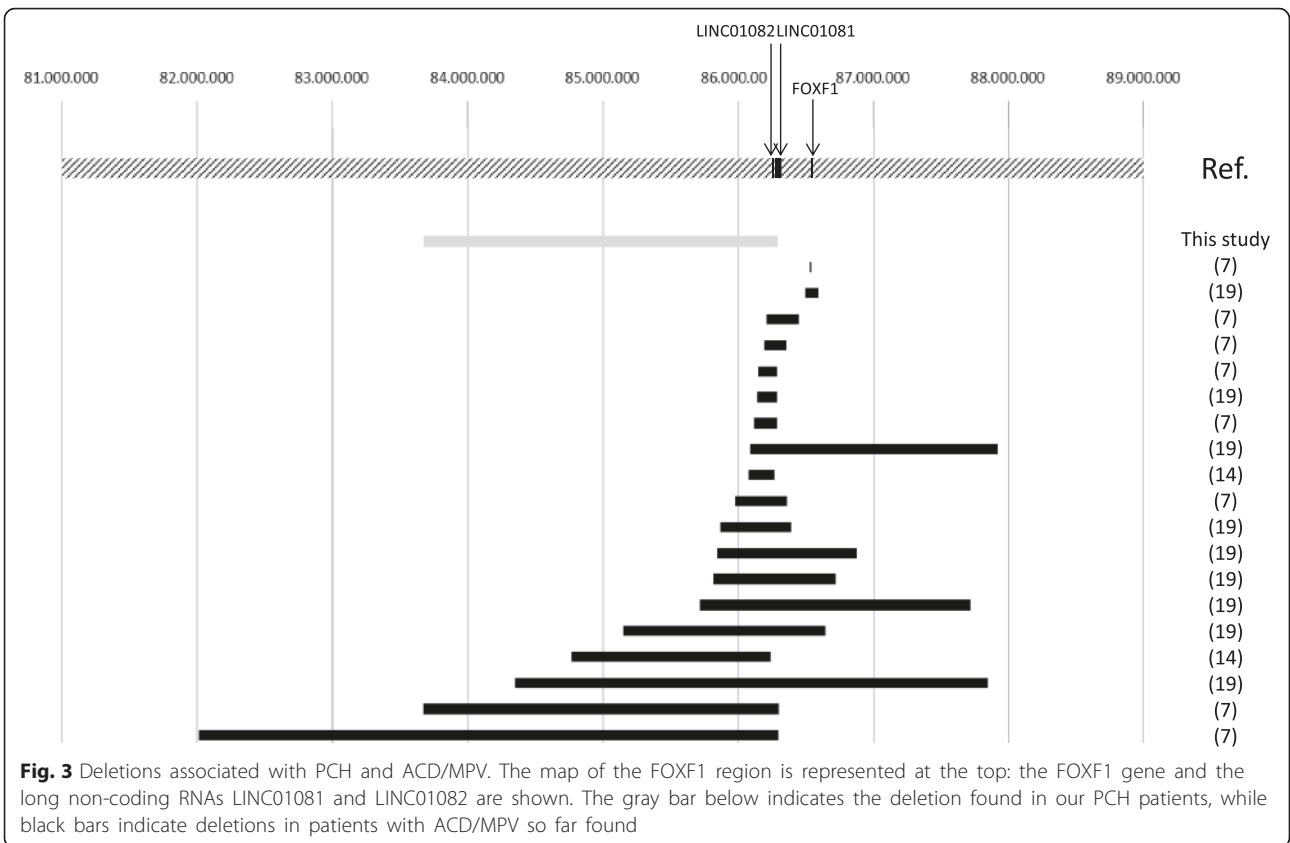
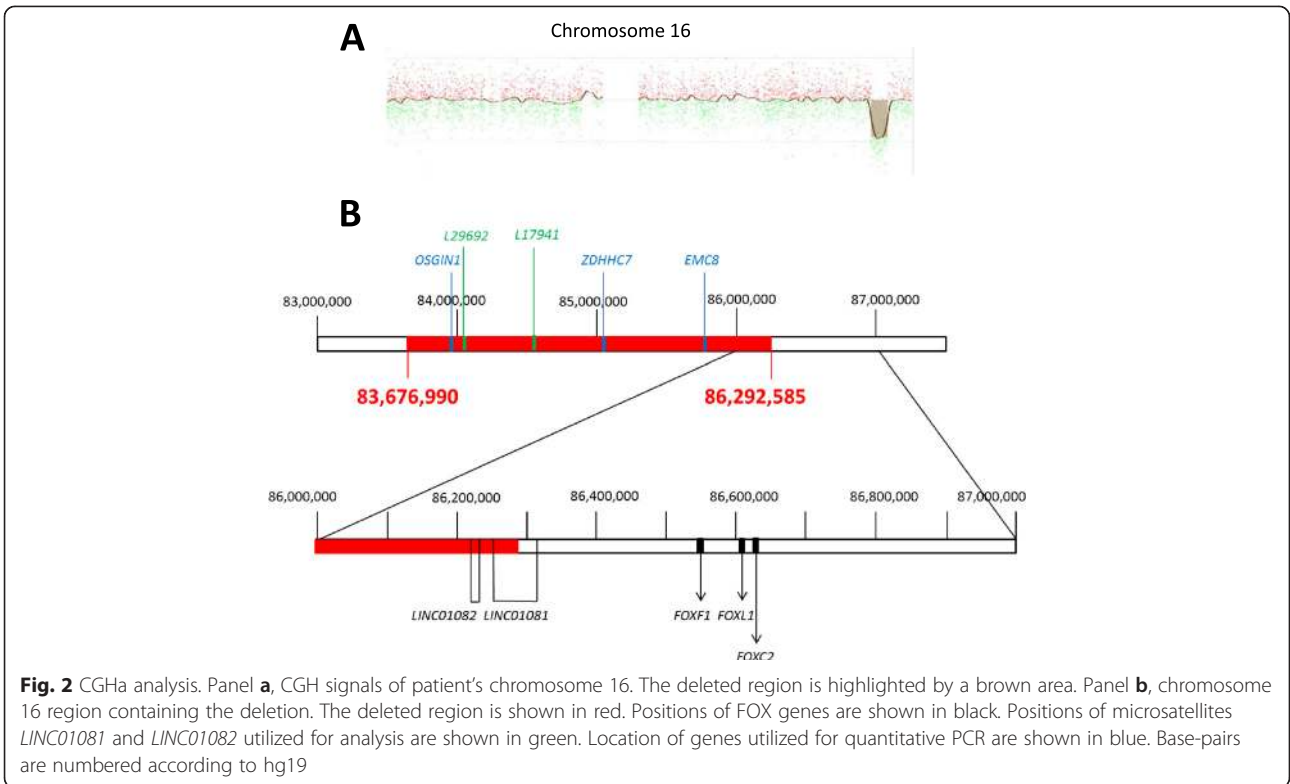
It is known that the 16q23.3q24.1 region could be subjected to parental imprinting; in fact, in ACD/MPV, the deletion always occurs in the maternal chromosome and the paternal allele is less expressed than the maternal one [10]. Thus, in order to test whether the 16q23.3q24.1 deletion occurred in the maternal chromosome, a microsatellite analysis was conducted inside the deletion. As shown in Fig. 4, analysis of L17941 and L29692 microsatellites indicate that the deletion indeed occurs in the maternal chromosome.

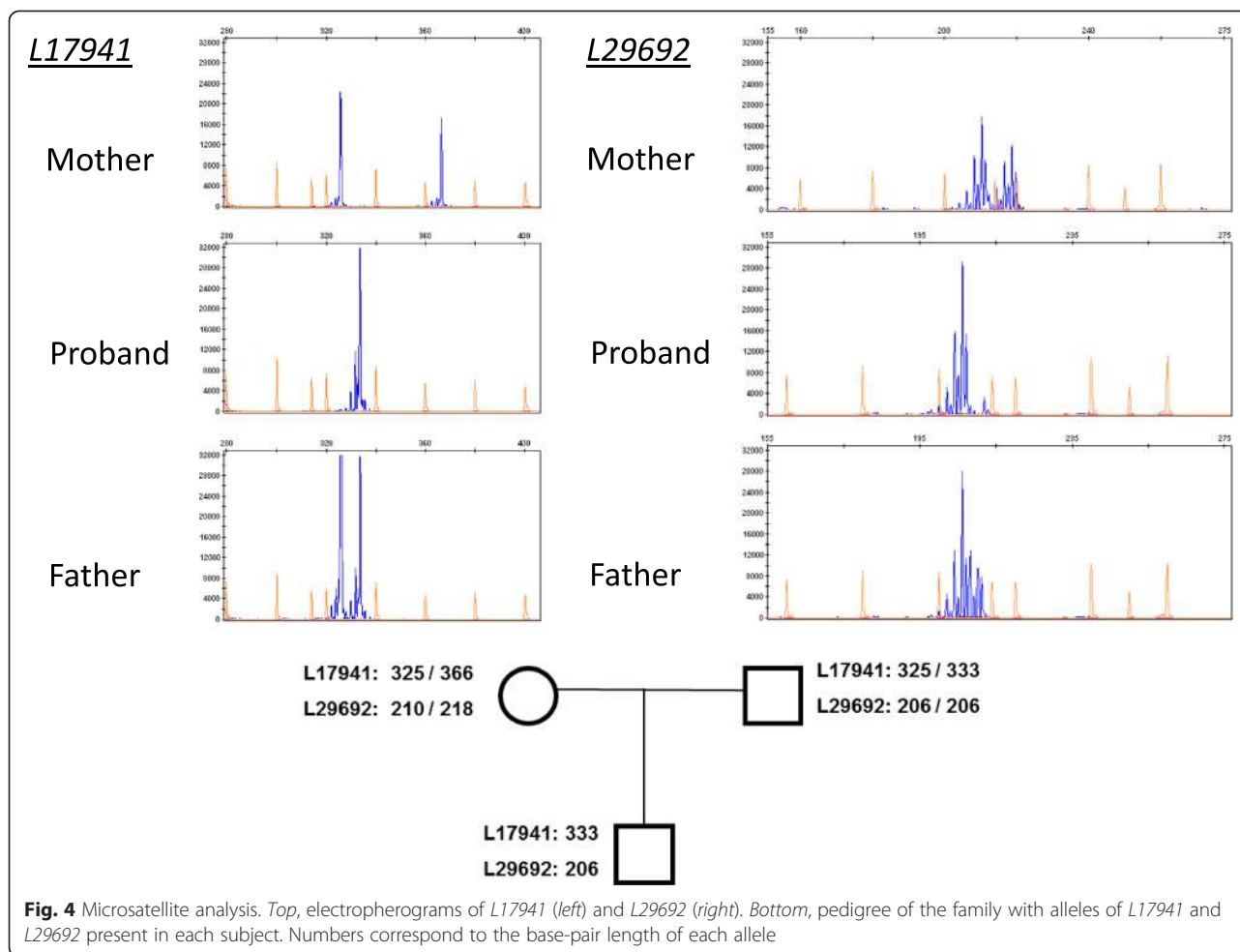
Conclusion

The PCH histopathologic lungs features show a crowded and congested alveolar capillary bed without pulmonary venous misalignment and lymphatic alteration. The alveolar capillary expansion is usually associated with intra-alveolar hemorrhage [4, 5]. Based upon our microscopical findings, our patient suffers of PCH with structural abnormalities in preacinar and intra-acinar pulmonary arteries, consistent with the morphologic characteristics of persistent pulmonary hypertension of the newborn. The radial-alveolar counts were reduced, reflective of decreased alveolarization.

PCH is considered an underestimated pathology, because it may mimic idiopathic pulmonary arterial hypertension, pulmonary veno-occlusive disease, atypical interstitial lung disease, misalignment of lung vessel and alveolar capillary dysplasia or congenital pulmonary lymphangectasia [2, 11–13]. Based on familial occurrence, it is possible that congenital forms of this disease may have a genetic determination. Best et al. [6] have shown the involvement of *EIF2AK4* gene mutations in PCH pathogenesis. However, it is likely that *EIF2AK4* mutations do not account for all cases of PCH [2].

In our case, CGHa revealed a chromosome 16q23.3-q24.1 deletion that disrupts the distant *FOXF1* transcriptional enhancer, which maps about 257 kb upstream to the *FOXF1* gene. In this enhancer are located two





lncRNAs: *LINC01081* and *LINC01082* [7]. It has been demonstrated that *in vitro* abolition of *LINC01081* by siRNA, reduces *FOXF1* expression [14]. Thus, our data could suggest that the disruption of the *FOXF1* gene transcriptional enhancer induces cell proliferation and migration. This is consistent with other findings indicating *FOXF1* as an oncosuppressor gene [15, 16]. The analysis of fusion progeny between mesenchymal stem cells and lung cancer cells has recently demonstrated that *FOXF1* significantly reduced the growth rate and expression levels of proteins regulating the cell cycle [17]. Accordingly, previous investigations have proposed PCH to be a lung endothelial neoplasia [18]. Based upon the relevance of *LINC01081* on *FOXF1* expression regulation, our data could suggest that the disruption of this long non-coding RNA can lead to architectural changes in pulmonary vessels, resulting in neonatal-onset PCH.

Consent

This study has been performed in accordance with the Helsinki declaration. Written informed consent was obtained from the parents of the patient for publication of

this case report. A copy of the written consent is available for review by the Editor of this journal.

Additional file

Additional file 1: Table S1. Genes involved in our PCH patient deletion. (DOCX 18 kb)

Competing interests

The authors declare not have competing interests.

Authors' contributions

PDL, CGH analysis and contribution in writing the paper; AF, Microsatellites analysis; FB, Quantitative PCR; CP, (Cinzia Puppini) interpretation of molecular data and contribution in writing the paper; GDM, Histology and immunohistochemistry; CP, (Carla Pittini) clinical evaluation and counselling; LC, Clinical evaluation; SP, histology, immunohistochemistry and contribution in writing the paper; GD, Conception of the study and writing the paper. All authors read and approved the final manuscript.

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Author details

¹Dipartimento di Medicina di Laboratorio, Azienda Ospedaliero-Universitaria S. Maria della Misericordia, Udine, Italy. ²Dipartimento di Scienze Mediche e Biologiche, Università di Udine, Piazzale Kolbe 4, 33100 Udine, Italy.

³Dipartimento Materno-infantile, Azienda Ospedaliero-Universitaria S. Maria della Misericordia, Udine, Italy.

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