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

Patrizia Dello Russo1, Alessandra Franzoni1, Federica Baldan2, Cinzia Puppin2, Giovanna De Maglio1, Carla Pittini3, Luigi Cattarossi3, Stefano Pizzolitto1 and Giuseppe Damante1,2*

Abstract

Background: Pulmonary capillary hemangiomatosis (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 hemangiomatosis 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 hemangiomatosis, Chromosomal abnormalities, Deletion, Gene regulation

Background

Pulmonary capillary hemangiomatosis (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 CGHAs. 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

© 2015 Dello Russo et al. Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated.
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 hypodiafania of the right hemithorax. Blood tests were negative for infection. Ultrasound revealed a normal brain anatomy, with mild hyperechoic perivenricular left rear. Ultrasoundography 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 autopic lung samples were fixed in 4 % formalin for about 24 h. Fixed tissue was then paraffin embedded and 4 μm slides were stained with hematoxilin 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 QIApp 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 OSGINI, 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-CTGGGTACTCTTCT TGTGACA-3’;
Reverse: 5’-CTCTCTCCCCACATGGTG-3’.
L29692, Forward: 5’-6FAM-TGTGTGTCTTCTTG 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 were 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 intraalveolar 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
**Fig. 2** CGHa analysis. Panel a, CGH signals of patient’s chromosome 16. The deleted region is highlighted by a brown area. Panel b, chromosome 16 region containing the deletion. The deleted region is shown in red. Positions of FOX genes are shown in black. Positions of microsatellites LINC01081 and LINC01082 utilized for analysis are shown in green. Location of genes utilized for quantitative PCR are shown in blue. Base-pairs are numbered according to hg19.

**Fig. 3** Deletions associated with PCH and ACD/MPV. The map of the FOXF1 region is represented at the top: the FOXF1 gene and the long non-coding RNAs LINC01081 and LINC01082 are shown. The gray bar below indicates the deletion found in our PCH patients, while black bars indicate deletions in patients with ACD/MPV so far found.
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 Puppin) 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.

Acknowledgements
This work has been funded by the Interreg SIGN (Slovenian Italian Genetic Network) project. We thank Catia Mio for the critical review of the manuscript.
Author details
1 Dipartimento di Medicina di Laboratorio, Azienda Ospedaliero-Universitaria S. Maria della Misericordia, Udine, Italy. 2 Dipartimento di Scienze Mediche e Biologiche, Università di Udine, Piazzale Kolbe 4, 33100 Udine, Italy. 3 Dipartimento Materno-infantile, Azienda Ospedaliero-Universitaria S. Maria della Misericordia, Udine, Italy.

Received: 4 July 2015 Accepted: 4 October 2015
Published online: 13 October 2015

References
1. Folkman J, Klagsbrun M. Angiogenic factors. Science. 1987;235:442–7.
2. Langleben D, Montreal MD. Pulmonary capillary hemangiomatosis: the puzzle takes shape. Chest. 2014;145:197–9.
3. Wirbelauer J, Hebestreit H, Eugene AM, Mark J, Speer CP. Familial pulmonary capillary hemangiomatosis early in life. Case Rep Pulmonol. 2011. doi:10.1155/2011/827591.
4. Simonneau G, Gatzoulis MA, Adatia I, Celermajer D, Denton C, Ghofrani A, et al. Clinical classification of pulmonary hypertension. J Am Coll Cardiol. 2013;62:D34–41.
5. Oviedo A, Abramson LP, Worthington R, Dainauskas JR, Crawford SE. Congenital pulmonary capillary hemangiomatosis: report of two cases and review of literature. Pediatr Pulmonol. 2003;36:253–6.
6. Best DH, Sumner KL, Austin ED, Chung WK, Brown LM, Borczuk AC, et al. EIF2AK4 mutations in pulmonary capillary hemangiomatosis. Chest. 2014;145:231–6.
7. Szafrański P, Dharmadhikari AV, Brosens E, Gurha P, Kołodziejska KE, Zhishuo O, et al. Small noncoding differentially methylated copy-number variants, including IncRNA genes, cause a lethal lung developmental disorder. Genome Res. 2013;23:23–33.
8. Wagenvoort CA. Misalignment of lung vessels: a syndrome causing persistent neonatal pulmonary hypertension. Hum Pathol. 1986;17:727–30.
9. Sen P, Thakur N, Stockton DW, Langston C, Bejjani BA. Congenital pulmonary capillary dysplasia (ACD). Pediatr Pulmonol. 2004;39:232–6.
10. Sen P, Gerychova R, Janku P, Jezova M, Navarro C, et al. A familial case of alveolar capillary dysplasia with misalignment of pulmonary veins supports paternal imprinting of FOXL1 in human. Eur J Hum Genet. 2013;21:474–7.
11. Hung S-P, Huang S-H, Wu C-H, Chen W-C, Kou K-E, Wang N-K, et al. Misalignment of lung vessels and alveolar capillary dysplasia: a case report with autopsy. Pediatr Neonatol. 2011;52:232–6.
12. Michalsky MP, Avicente E, Freyk G, Sue H, Dick T, Caniano DA. Alveolar capillary dysplasia: a logical approach to a fatal disease. J Pediatr Surg. 2005;40:1100–5.
13. Nouri-Merchaoui S, Mahdoumi N, Yacoubi M-T, Seboul H. Congenital pulmonary lymphangiectasis: an unusual cause of respiratory distress in neonates. Arch Pediatr. 2012;19:408–12.
14. Szafrański P, Dharmadhikari AV, Wambach JA, Towe CT, White FV, Grady RM, et al. Two deletions overlapping a distant FOXL1 enhancer unravel the role of IncRNA LINC01081 in etiology of alveolar capillary dysplasia with misalignment of pulmonary veins. Am J Hum Genet. 2014;164A:2013–9.
15. Tamura M, Sasaki Y, Koyama R, Takeda K, Idogawa M, Tokino T. Forkhead transcription factor FOXL1 is a novel target gene of the p53 family and regulates cancer cell migration and invasiveness. Oncogene. 2014;23:4637–46.
16. Lo PK, Lee JS, Liang X, Han L, Mori T, Fackler MJ, et al. Epigenetic inactivation of the potential tumor suppressor gene FOXL1 in breast cancer. Cancer Res. 2010;70:6947–58.
17. Wei HJ, Nickoloff JA, Chen WH, Liu HY, Lo WC, Chang YT, et al. FOXL1 mediates mesenchymal stem cell fusion-induced reprogramming of lung cancer cells. Oncotarget. 2014;5:9514–29.
18. Wagenvoort CA, Beetstra A, Spijker J. Capillary haemangiomatosis of the lungs. Histopathology. 1978b:401–6.
19. Stankiewicz P, Sen P, Bhatt SS, Storer M, Xia Z, Bejjani BA, et al. Genomic and genetic deletions of the FOXL1 gene cluster on 16p24.1 and inactivating mutations of FOXL1 cause alveolar capillary dysplasia and other malformations. Am J Hum Genet. 2009;84:780–91.