

## CASE REPORT

# Type 1 neurofibromatosis complicated by pulmonary artery hypertension : a case report

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**Abstract :** We describe the case of a patient with neurofibromatosis type 1 (NF1) complicated by severe pulmonary arterial hypertension (PAH) ; only seven cases have been reported on this association so far, and PAH seems to be related to the vascular involvement of neurofibromatosis. The histology of our patient's lung tissue showed thickening of arteries and veins by medial and/or intimal hypertrophy and fibrosis. In order to exclude a familiar PAH, the analysis of the bone morphogenetic protein receptor 2 gene was carried out, but no mutations were found.

On the basis of histological findings and of the results of genetic study we believe that PAH was a complication of NF1 in our patient and we suggest to screen patients with NF1 for the presence of PAH by means of trans-thoracic echocardiogram. *J. Med. Invest.* 54 : 354-358, August, 2007

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## INTRODUCTION

Neurofibromatosis type 1 (NF1), also known as von Recklinghausen's disease, is an autosomal dominant disease with complete penetrance and extremely variable expression ; the incidence is approximately of 1 in 4000 live births (1). The predominant clinical hallmarks of NF1 are *café-au-lait* macules, neurofibromas, Lisch nodules of the iris and axillary freckling. Other clinical manifestations are abnormalities of the cardiovascular, gastrointestinal, renal and endocrine systems, facial and body disfigurement, cognitive deficit, and malignancies of the peripheral nerve sheath and central nervous system (2). The

NF1 gene on chromosome 17q11.2 spans more than 350 kb of genomic DNA and contains 60 exons (1). The NF1 gene product neurofibromin is a GTPase activating protein for members of p21ras (Ras) protein family. Since neurofibromin negatively regulates Ras output by accelerating the conversion of active Ras-GTP to inactive Ras-GDP, loss of neurofibromin function leads to downstream cell growth activation (3). Recent findings report that NF1 product modulates also protein kinase B (Akt), an important regulator of cell proliferation and survival (4).

Pulmonary artery hypertension (PAH) is a disease of the small pulmonary arteries characterized by vessel proliferation and vessel wall remodelling. It results in a progressive increased in pulmonary vascular resistance and, ultimately in right ventricular failure and death. A diagnosis of primary (or idiopathic) pulmonary artery hypertension (PAH) is made when no known risk factors or other causes

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are identified (5). It is a progressive disease that usually leads to death within 5 years from the diagnosis.

Familial pulmonary hypertension is a rare (6% of all cases) autosomal dominant disorder with reduced penetrance, that has been mapped to a 3-cM region on chromosome 2q33. It is characterized by monoclonal plexiform lesion on proliferating endothelial cells. The disease can occur from infancy throughout life (6). Mutations in the gene encoding for the bone morphogenetic protein type II receptor (BMPR2) have been identified in 72% of affected families and 26% of apparently sporadic cases. The mutations are widely dispersed throughout the gene (7). BMPs are members of the transforming growth factor (beta) superfamily and affect intracellular signalling via Smads and mitogen-activated protein kinases (8).

We report here the case of a patient with von Recklinghausen disease complicated by severe PAH. The analysis of the BMPR2 gene did not show the presence of any mutation.

## CASE REPORT

A 51-year-old Caucasian woman was admitted to our hospital because of progressive dyspnea on exertion and hyposthenia (NYHA class III). She had a history of hypertension since the age of 20.

Physical examination showed many cutaneous neurofibromas on her trunk, and multiple *café au lait* spots, the biggest one (4 cm) on her left wrist. She had neither peripheral or central cyanosis nor clubbing. Her blood pressure was 180/105 mm Hg and heart rate 110 beats/min; there were inspiratory crackles at the right lung and a raised jugular venous pulse. Examination of the heart was normal, the liver was palpable at the costal margin and there was a swelling on the right clavicle region.

Laboratory investigations revealed high ESR (57 mm/h; *nv* <35) and CRP (37 mg/L *nv* <5). Full blood count showed iposideremic microcytic anaemia (Hb 9,2 gr/dl, MCV 74 fl, MCH 24 pg). Autoantibodies were negative; liver and renal functions and coagulation tests were normal. Serologic testing for HIV was negative. Arterial blood gas tensions (on room air) were as follows: PH 7,43, PCO<sub>2</sub> 27 mmHg, PO<sub>2</sub> 72 mmHg, HCO<sub>3</sub> 18 mmol/L, O<sub>2</sub> Sat 90%.

Neck ultrasonography showed a mass of 5 cm at the right supraclavicular region: the needle biopsy of

the mass was not diagnostic. Chest X-ray showed bilateral pleural effusion; chest computed axial tomography showed also two nodular lesions with irregular margins at the basal region of the right lung with pleural infiltration and a large mass in upper mediastinum. Bronchoscopy was negative. An abdomen ultrasonography was normal. A transthoracic echocardiogram revealed concentric hypertrophy of left ventricle, enlarged left atrium (48 mm), interventricular septum of 13 mm and a right ventricular systolic pressure of 35 mmHg (the peak of gradient through a mild tricuspid regurgitation was 30 mmHg + 5 mmHg of right atrial pressure, by the normal size of a collapsing vena cava, were added to estimate right ventricular systolic pressure).

The patient was unable to carry out the spirometric test.

Biopsy of the mediastinum mass was compatible with schwannoma. The morphological examination of pulmonary tissue was consistent with pulmonary hypertension. The main histopathological features included the presence of focal areas of necrosis, the occurrence of numerous alveolar macrophages containing hemosiderin, and a diffuse alteration of vessel walls. Both arteries and veins appeared thickened by medial and/or intimal hypertrophy and fibrosis (Fig. 1). Infiltration of S100+ neurofibromatosis cells was not evident.

On the basis of the histopathological findings and of the worsening of dyspnoea, after two months we performed a second echocardiogram, that showed severe dilation of right chambers, with moderate tricuspid regurgitation, dilatation of vena cava and severe right ventricular systolic pressure (115 mmHg).

A cardiac catheterization confirmed the presence of severe high PAH (108/39 mmHg; mean, 65 mmHg), mean pulmonary wedge pressure 11 mmHg. After PGI<sub>2</sub> infusive test (vasoreactivity testing), pulmonary vascular resistance remained unchanged.

Plasma N-terminal pro-B type natriuretic peptide (proBNP) level was 12,6 pmol/l (*nv* < 14,8 pmol/L). However its levels markedly increased after three and nine months: 40,8 pmol/l and 472 pmol/l respectively; cardiac troponin T was normal.

Since the patient's grandmother died of lung failure, the genetic analysis of BMPR2 was carried out in order to exclude a familiar PAH.

Based on the diagnosis of pulmonary hypertension, she was started on diuretics, warfarin and bosentan therapy (62,5 mg twice day for 4 weeks and then 125 mg twice daily). NYHA class as well

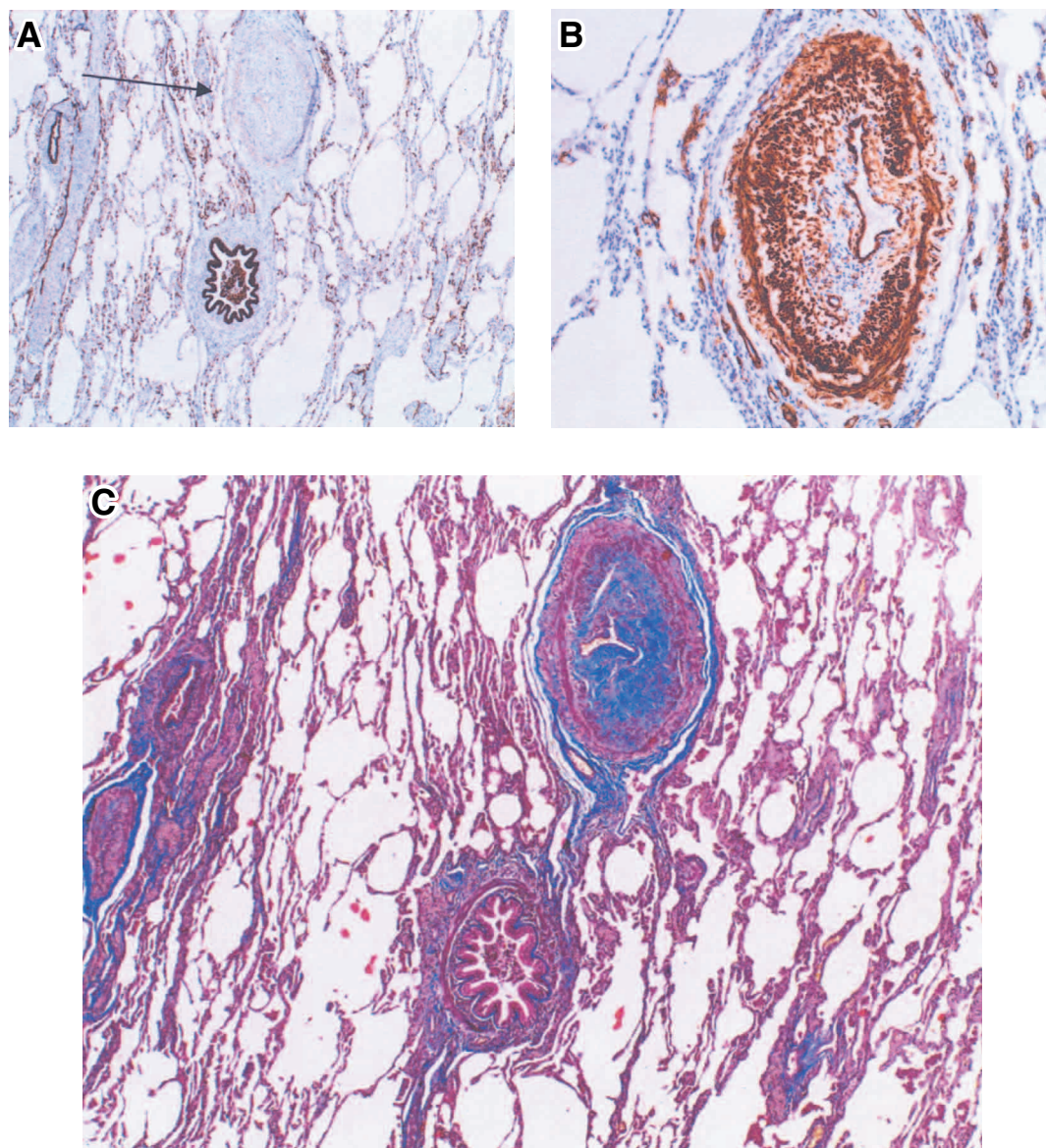


Fig. 1. Pulmonary artery with medial hypertrophy and marked intimal fibrosis. (A) Anti-cytokeratin immunostaining (arrow). (B) Alpha-smooth-muscle actin and (C) trichrome staining.

as the 6 minutes walking test remained stable for nearly two and half years but recently, because of worsening of dyspnoea, sildenafil therapy has been introduced.

#### *Analysis of the BMPR2 gene*

DNA was extracted from 10 ml of whole-blood with Puregene Genomic DNA purification kit (GENTRA systems, Minneapolis, USA) after patient's informed consent was obtained. Exons 1 to 13 of BMPR2 gene were amplified by polymerase chain reaction (PCR) from 100 ng of genomic DNA (Gene-amp High Fidelity PCR System Kit, Applied Biosystem, California, USA). The sequence of the primers used has been already reported elsewhere (6). Since we had some difficulties in amplifying exon 12,

we wanted to confirm the data obtained by cDNA synthesis. To this aim, RNA was extracted from peripheral blood cells using TriReagent extraction kit (Sigma Corporation, St Louis, Missouri, USA) and retrotranscribed with the SuperScript III first-Strand Synthesis System (Invitrogen, Groningen, The Netherlands) using oligo(dt) primers. cDNA was then amplified with the appropriate primers. BMPR2 cDNA was purified with QIAquick gel extraction kit (QIAGEN).

The PCR fragments obtained from genomic DNA and cDNA were sequenced using MWG Biothec service (Ebersberg, Germany) and compared with the published sequence of the gene and of cDNA. We did not find any mutation in the exons 1 to 13 of the BMPR2 gene.

## DISCUSSION

We report the case of a patient with neurofibromatosis type 1 and PAH. Only seven cases of PAH in von Recklinghausen's disease have been reported so far (9-13). In all reports PAH seems to be related to the vascular involvement of neurofibromatosis although histology was described only in one case.

In our patient all the conditions associated with PAH such as connective tissue diseases, chronic lung disease, congenital or acquired heart diseases, HIV infection, drug and toxins, hemoglobinopathies, myeloproliferative disorders, thromboembolism were excluded. Vasculopathy associated to neurofibromatosis was the probable cause of PAH, however we wanted to exclude the possibility of mutations in the *BMPR2* gene since there was a suspected case of PAH in her grandmother. Indeed in the past year mutations in the *BMPR2* gene have been identified in a high proportion of cases of familial and sporadic PAH. BMP type II receptor is located in lipid rafts, including caveolae, of pulmonary endothelium *in vivo* and *in vitro* (14). Caveolae are vesicular organelles that are particularly abundant in cells of cardiovascular system including endothelial cells, smooth muscle cells and fibroblasts (4). Caveolin knockout mice display dramatic abnormalities in the cardiovascular system, such as pulmonary hypertension and cardiac hypertrophy (15). Neurofibromin, the protein encoded by the *NF1* gene has been detected in the endothelial cell layer of rat cerebral vessels, renal arteries, and aorta by immunohistochemistry. Neurofibromin expression was also detected in smooth muscle layer of the aorta (16). Recently some authors have reported that neurofibromin modulates Akt, an important regulator of cell proliferation and survival, and binds to caveolin-1 (4). Therefore the interplay of neurofibromin, caveolin and akt may play a pivotal role in the pathogenesis of PAH in patients with *NF1*.

In conclusion, based on the histological findings of the lung tissue and on the genetic analysis of *BMPR2*, we think that PAH was a complication of *NF1* in our patient and suggest that patients with *NF1* need to be evaluated with trans-thoracic echocardiogram for the presence of PAH, even if it has low sensibility to screen initial forms.

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