LEARNING THE ROPES: PULMONARY HYPERTENSION

Genetic architecture of pulmonary hypertension: From gene discovery to genetic counseling

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The genetic architecture of group 1 pulmonary hypertension (PH), including pulmonary arterial hypertension (PAH) and pulmonary veno-occlusive disease (PVOD) has deeply changed during the last 20 years. *BMPR2* (coding for bone morphogenetic protein receptor type-2), the first and major gene linked to heritable PAH, was identified in 2000 through genetic linkage studies in several large multiplex families¹. Whole exome and whole genome sequencing in affected families or in large populations of cases and control subjects allowed identification of a few predisposing genes involved less frequently in heritable PAH.

The BMP (bone morphogenetic protein) pathway genes are the major genetic actors of non-syndromic heritable PAH since pathogenic loss-of-function variants were identified at the heterozygous state in affected PAH patients, confirming that heritable PAH is transmitted as an autosomal dominant disease with incomplete penetrance (i.e. % of mutation carriers developing the disease), because only 14% of male mutant carriers and 42% of female carriers will develop the disease². *BMPR2* is a type II receptor of BMP ligands and dimerizes with *ALK1* (Activin receptor-like kinase 1) and the co-receptor *endoglin* in endothelial cells, and the complex activates intracellular signaling and nuclear transcription of target genes through signaling intermediates that are *Smad1/5/8* and the coreceptor *Smad4*³. Indeed, pathogenic variants of *Smad9*, encoding

Smad8, were also rarely reported in PAH cases^{4,5}. Rendu-Osler disease (hereditary hemorrhagic telangiectasia, HHT), a hereditary vascular disease presenting with telangiectasias, epistaxis and visceral arterio-venous malformations, was shown to be linked to two major genes, *Endoglin* and *ACVRL1* (encoding *ALK1*)^{6,7}. PH can develop in HHT patients by arteriovenous malformations causing left-to-right shunts and high-throughput heart failure, but precapillary PAH can also develop with histological remodeling similar to idiopathic PAH. In this latter case *ACVRL1* was identified as the gene of PAH complicated HHT⁸. Other genes of the BMP pathway have been involved in heritable PAH such as *Smad9* in which rare pathogenic variants were found in PAH families⁴. Heterozygous loss-of-function (LOF) variants of *GDF2* encoding BMP9 (Bone morphogenetic protein 9) were initially described as responsible for a particular form of HHT and a homozygous child carrying a LOF variant of this gene was reported with severe PAH^{9,10}. Finally, *GDF2* heterozygous pathogenic variants were shown to be associated with PAH in a large whole genome sequence-based association study¹¹. These results were subsequently confirmed in European and Asian patients^{5,12}. A close paralog of *GDF2*, *BMP10*, was also investigated as a candidate gene and found mutated in rare cases in two studies^{5,13}.

The systematic search for gene mutations, has shown the presence of pathogenic variants in approximately 20% of sporadic PAH whereas this proportion reaches 80% in familial cases, mainly represented by *BMPR2*^{5,14}. *BMPR2* and *ACVRL1* mutation carriers develop PAH at an earlier age than mutation non-carriers in both sexes, and the age at death is also younger, both parameters varying among studies and the genes involved¹⁵. A follow-up study showed that in a quarter of asymptomatic *BMPR2* mutation carriers, mild or exercise-induced pulmonary hypertension could be detected and additional cases were detected during follow-up, yielding a PAH yearly incidence of 2.3%¹⁶.

Another group of heritable PAH is due to LOF mutations occurring in three genes important for lung development. These genes are *TBX4*, *SOX17*, and *KDR* (coding for VEGFR2/FLK1). The *TBX4* gene encodes a transcription factor important for lung and bone development, T-box transcription factor *TBX4*. Heterozygous LOF mutations lead to the small patella syndrome, an autosomal dominant skeletal syndrome¹⁷. In a small proportion of *TBX4* mutation carriers, PAH develops at various ages of onset, from neonates to adult patients^{18–20}. Clinical presentation, hemodynamic parameters and histological lesions are similar to idiopathic PAH. Some elements suggest a parenchymal lung involvement of developmental origin in affected patients, such as abnormal distal lung development, small size of the lung, and low diffusing capacity for carbon monoxide adjusted for hemoglobin (D_{LCO}), although some *TBX4* mutation carriers seem to have an exclusive vascular involvement²⁰.

The *SOX17* gene, coding for the transcription factor SOX-17, was identified as a PAH gene through a whole genome sequencing (WGS) gene-based association study and a single nucleotide polymorphism based genome wide association study^{11,21}. A whole exome sequencing (WES) study in PAH associated with congenital heart diseases clearly indicated the involvement of this gene¹¹. Indeed, *SOX17* is expressed in arterial endothelial cells of the embryonic vasculature and conditional deletion of *Sox17* in mesenchymal progenitor cells

demonstrates that *Sox17* is required for normal vasculature morphogenesis and normal cardiac morphogenesis.

Preliminary evidence for a responsibility of the *KDR* gene encoding VEGFR2, the main receptor for vascular endothelial growth factor A (VEGF-A) was initially suggested by a WGS-based association study¹¹. Subsequently, two *KDR* pathogenic variants were found in a prospective study in PAH patients from two different families²². In both families, affected mutation-carriers had a reduced D_{LCO} , from 19 to 35%, consistent with parenchymal lung involvement observed on high-resolution CT scan. A reduced D_{LCO} is also observed but to a lesser extent in non-affected mutation carriers. This parameter can therefore be used as a biomarker of the disease in the context of this genetic predisposition²². A subsequent association study based on WES showed additional *KDR* pathogenic variants in PAH²³. Since expression of *KDR* is high in human embryonic endothelial cells, and VEGF signaling has a major role for proper blood vessel and epithelium-capillary bilayer formation and maintenance, it is likely that defect in VEGF signaling resulting from *KDR* haploinsufficiency might impair lung vasculature development and alveolar maintenance during postnatal life (reviewed in [22]).

Exome sequencing of a large family has identified a likely pathogenic variant of the *KCNK3* gene encoding a pH-sensitive potassium channel, and segregating in all affected cases of the family²⁴. The functional effects of the *KCNK3* variants found in a few PAH families were tested in vitro, showing a decreased function of the mutated channel. The pulmonary expression of the channel was also shown to be reduced in human PAH and experimental model of PAH in rats²⁵. Homozygous deficient rats for *Kcnk3*, showed more severe PAH when challenged by hypoxia or monocrotaline²⁶. A severe and early PAH was observed in a child homozygous for a *KCNK3* likely pathogenic variant from a highly consanguineous family²⁷. However, from clinical testing data, *KCNK3* seems very rarely involved in PAH.

More genes were identified through WES or WGS gene-based association studies. These genes are aquaporin (*AQP1*), encoding a water channel protein, *ATP13A3* encoding a protein involved in cation transport across membranes¹¹ and *ABCC8* encoding an ABC protein transporting molecules across cellular membranes²⁸. The *FBLN2* and *PDGFD* genes were also identified through the same strategy in various forms of PAH²³.

Pulmonary veno-occlusive disease (PVOD) is either sporadic, mainly due to tobacco exposure or chemotherapy by alkylating agents, or hereditary (hPVOD) linked to biallelic loss of function mutations of the *EIF2AK4* gene²⁹. This lung vascular disease belongs to group 1.6 of PAH, that includes PAH with overt features of venous/capillaries involvement³⁰. Clinically, PVOD can be misdiagnosed as PAH because there is no hemodynamic difference. Some clues come from abnormal chest imaging ground glass opacities, septal lines and lymphadenopathy and from a decreased D_{LCO} ³¹. hPVOD, is transmitted as an autosomal recessive disease and age at onset is far younger than in sporadic forms (26 vs 60 yrs)³². Genetic analysis showing biallelic *EIF2AK4* mutations is able to correct the diagnosis. Vascular remodeling predominates on veins and veinules with intimal fibrosis and medial hypertrophy, muscular hyperplasia of interlobular septal veins, but arterial remodeling and microvascular muscularization are also observed³³. Capillary

hemangiomatosis (PCH)-like foci are also observed and explain the term pulmonary capillary hemangiomatosis that also designates the disease also linked to *EIF2AK4* mutations in its hereditary form³⁴. The evolution is extremely severe, and treatment used in PAH are contraindicated because of severe adverse effects.

Genetic counselling is a major contribution of gene identification in the care and support of carrier patients. Preimplantary diagnosis (PID) can be proposed to parents when there is a risk for the fetus to carry a mutation leading to a severe and intractable disease and if feasible, is preferred to prenatal diagnosis, especially if the mother is affected. The best example is hereditary PVOD because the penetrance is high, the disease very severe and one fourth of the offspring from two heterozygous parents will carry mutations on both alleles. PID was also performed in a case of *BMPR2* linked severe familial PAH³⁵.

Although some unexplained familial cases are likely linked to deep intronic mutations in known and sequenced genes, it is likely that new genes will be identified in extremely rare cases. The current important challenge is understanding the incomplete and sexually differentiated penetrance of PAH, the variable age of onset of the disease. A putative second hit of unknown nature remains to be identified.

Figure: Schematic representation of the genes involved in heritable pulmonary arterial hypertension and hereditary pulmonary veno-occlusive disease.

Only genes in bold characters are directly involved. The main components of the BMP pathway are depicted. Smad 8 is encoded by the *SMAD9* gene. *BMP9* is encoded by *GDF2*. *GCN2* (encoded by *EIF2AK4*) is involved in pulmonary veno-occlusive disease. Both the gene name and the protein name (between parentheses) are indicated when different. *ATP13A3* is a member of the P-type ATPase family of proteins that transport a variety of cations across membranes. *ABCC8* is a member of the superfamily of ATP-binding cassette (ABC) transporters. Aquaporin 1 is a small integral membrane protein that functions as a water channel protein.