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Genetics and Genomics of Pulmonary Arterial Hypertension

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Major discoveries have been obtained within the last decade in the field of hereditary predisposition to pulmonary arterial hypertension (PAH). Among them, the identification of *bone morphogenetic protein receptor type 2* (*BMPR2*) as the major predisposing gene and *activin A receptor type II-like kinase-1* (*ACVRL1*, also known as *ALK1*) as the major gene when PAH is associated with hereditary hemorrhagic telangiectasia. The mutation detection rate for the known genes is approximately 75% in familial PAH, but the mutation shortfall remains unexplained even after careful molecular investigation of these genes. To identify additional genetic variants predisposing to PAH, investigators harnessed the power of next-generation sequencing to successfully identify additional genes that will be described in this report. Furthermore, common genetic predisposing factors for PAH can be identified by genome-wide association studies and are detailed in this paper. The careful study of families and routine genetic diagnosis facilitated natural history studies based on large registries of PAH patients to be set up in different countries. These longitudinal or cross-sectional studies permitted the clinical characterization of PAH in mutation carriers to be accurately described. The availability of molecular genetic diagnosis has opened up a new field for patient care, including genetic counseling for a severe disease, taking into account that the major predisposing gene has a highly variable penetrance between families. Molecular information can be drawn from the genomic study of affected tissues in PAH, in particular, pulmonary vascular tissues and cells, to gain insight into the mechanisms leading to the development of the disease. High-throughput genomic techniques, on the basis of next-generation sequencing, now allow the accurate quantification and analysis of ribonucleic acid, species, including micro-ribonucleic acids, and allow for a genome-wide investigation of epigenetic or regulatory mechanisms, which include deoxyribonucleic acid methylation, histone methylation, and acetylation, or transcription factor binding. (J Am Coll Cardiol 2013;62:D13–21) © 2013 by the American College of Cardiology Foundation

Genetics of Pulmonary Hypertension

Hereditary predisposition to pulmonary arterial hypertension: from major genes to associated single nucleotide polymorphisms. Over 300 independent *BMPR2* mutations (coding for a type II receptor member of the transforming growth factor [TGF]-β family) have been identified that account for approximately 75% of patients with a known family history of pulmonary arterial hypertension (PAH), and up to 25% of apparently sporadic cases have now unequivocally established defects in this gene as the major genetic determinant underlying PAH (1). Pathogenic mutations in the type I receptor *ACVRL1* and, at a significantly lower frequency, the type III receptor endoglin in multiple kindreds cause PAH associated with hereditary hemorrhagic telangiectasia (HHT) (2). Together, these observations support a prominent role for TGF-β family members in the development of PAH. Consequently, a series of

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Abbreviations and Acronyms

BMP = bone morphogenetic protein
CHD = congenital heart disease
GINA = Genetic Information Non-Discrimination Act
GSD = glycogen storage disease
HDAC = histone deacetylase
HHT = hereditary hemorrhagic telangiectasia
HPAH = heritable pulmonary arterial hypertension
IL = interleukin
IPAH = idiopathic pulmonary arterial hypertension
mRNA = messenger ribonucleic acid
miRNA = micro ribonucleic acid
PAEC = pulmonary artery endothelial cell
PAH = pulmonary arterial hypertension
PASMC = pulmonary artery smooth muscle cell
SNP = single nucleotide polymorphism
TGF = transforming growth factor

studies have adopted a candidate gene approach to delineate novel genetic variants by examining TGF-β receptors and effectors in patient cohorts without mutations in the known PAH genes. With conventional analytical techniques, Shintani et al. (3) identified a truncating mutation in the bone morphogenetic protein (BMP)-responsive gene *SMAD9* (p.C202X) in a panel of 23 Japanese cases. A second truncating mutation (p.R294X) has since been identified in another patient of Asian descent (4). A similar screen of the BMP-specific SMADs and SMAD4 described a series of 4 variants in 198 idiopathic pulmonary arterial hypertension (IPAH) patients. These variants in *SMAD1* (p.V3A), *SMAD4* (p.N13S; c.1448-6T>C), and *SMAD9* (p.K43E) were described as being of unknown significance due to their moderate effects on canonical downstream BMP-mediated signaling outcomes (5). The *SMAD9* variants are more compelling, because these data are supported by the development of clinical and histopathological features of pulmonary hypertension in a *Smad9* knock-out mouse model (6). More recently, 2 missense mutations of the type I receptor *BMPR1B* (p.S160N and p.F392L) were reported in a cohort of 43 IPAH patients. Subsequent functional and reporter assays suggested that these variants generated an induction of SMAD9 and augmentation of transcriptional activity indicative of a gain-of-function mechanism. Because the preceding studies, in conjunction with the *Smad9* mutant mouse model, suggest a molecular mechanism of haploinsufficiency for this gene, the observations described by Chida et al. (7) would seem to be contradictory and require further investigation on the functional level. Austin et al. (8) used whole exome sequencing to study a 3-generation family with multiple affected family members with PAH but no identifiable mutation in the known heritable pulmonary arterial hypertension (HPAH) genes and identified a novel gene for HPAH: *Caveolin-1* (*CAV1*). They also identified a de novo frameshift mutation in a child with IPAH. *CAV1* encodes a membrane protein of caveolae abundant in the endothelium and other cells of the lung. Caveolae are rich in cell surface receptors critical to initiation of a cellular signaling cascade such as the TGFβ superfamily, nitric oxide pathway, and G-protein coupled receptors. Aberrant signaling at the plasma membrane might be the mechanism for PAH pathogenesis. Their study demonstrates that mutations in *CAV1* are associated in rare cases with familial PAH and IPAH, and it could provide new insight into the pathogenesis of PAH.

Exome sequencing in another family with multiple affected family members without identifiable HPAH mutations was found to have a heterozygous novel missense variant in the potassium channel *KCNK3* (9). Analysis for additional familial PAH cases and IPAH cases identified 5 additional heterozygous novel missense variants. All 6 variants are located in highly conserved amino acids and are predicted to be damaging by in silico analysis. With transient transfection in COS-7 cells, whole patch clamp procedures demonstrated that each of the 6 mutations resulted in loss of function. Some, but not all, mutations were rescued by the phospholipase inhibitor, ONO RS-082. *KCNK3* encodes a pH-sensitive potassium channel in the 2-pore domain superfamily (10). It has been reported that this potassium channel is sensitive to hypoxia and plays a role in the regulation of resting membrane potential and pulmonary vascular tone (11–13). Identification of this gene as a cause of HPAH and IPAH and the possibility of rescuing specific mutations might provide a new target for PAH treatment.

Childhood-onset PAH shows some clinical and genetic differences from adult-onset PAH. The frequency of *BMPR2* mutations found in sporadic cases is far lower than in adult-onset PAH (14–16). Pulmonary hypertension is an uncommon complication in many genetic disorders, although in certain syndromes such as Down syndrome, PAH is more common (17). The increased risk for PAH with Down syndrome is due to left-to-right cardiac shunts; in addition, upper airway obstruction associated with obstructive sleep apnea might promote non-PAH pulmonary hypertension (18). Genetic syndromes more commonly but not necessarily associated with congenital heart disease (CHD) and pulmonary hypertension include DiGeorge syndrome, VACTERL syndrome, CHARGE syndrome, Scimitar syndrome (19), Noonan syndrome (20), and chromosomal anomalies associated with congenital diaphragmatic hernia. Genetic syndromes associated with pulmonary hypertension usually not associated with CHD include Adams-Oliver syndrome (21,22), neurofibromatosis type 1 (23,24), long QT syndrome, hypertrophic cardiomyopathy, Cantu syndrome (25), autoimmune polyendocrine syndrome (26),

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