Update in Pulmonary Vascular Diseases 2013

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As in previous years, growing interest and a better understanding of the complexities of pulmonary vascular diseases continue to evolve at a healthy pace. The breadth of underlying mechanisms and potential targets for various pulmonary vascular disorders continues to expand and, as a result, new therapeutic modalities are emerging as attested, for example, by the U.S. Food and Drug Administration (FDA) approval this year of two drugs, including one targeting a new pathway, in pulmonary arterial hypertension (PAH). In the tradition of the *Journal*, this review summarizes major scientific accomplishments in the field of pulmonary vascular disease. It is obviously a daunting task to be as optimally inclusive or detailed in covering the past year's research in this field and thus we regret any unintentional omissions. Indeed, the purpose of this review is to efficiently transmit, under thematic headings, rapidly evolving information in one concise conduit that can be used as a step stone for the reader who is then encouraged to refer to the primary sources for more in-depth reading.

Pulmonary Circulation and Gas Exchange

The principal function of the lung is to facilitate gas exchange between the environment and blood. Although important insight has been gained from the numerical modeling of gas transfer along the pulmonary capillary combined with functional measurements of steady-state gas transfer, direct assessment of the contribution of precapillary gas exchange has not been possible. However, Tabuchi and colleagues used intravital microscopy combined with multispectral oximetry to achieve two-dimensional temporal and spatial mapping profiles in the intact, ventilated murine lung (1). Their study revealed that about half of the oxygen taken up during transit through the lung occurs in precapillary vessels less than 30 μm in diameter. Interestingly, in mice with vascular remodeling as a result of chronic hypoxia, precapillary oxygen transfer was significantly attenuated, presumably as a consequence of thickened arterial walls. This work extends previous understanding related to the physiology of lung gas exchange and it provides insight into how that function is disrupted in disease.

On Inflammation, Cellular, and Molecular Mechanisms Contributing to Vascular Remodeling

The role of inflammation in the pathogenesis of PAH and other vascular occlusive diseases such as pulmonary veno-occlusive disease (PVOD) has not lost momentum in the past year. To test the cytolytic arm of the cellular immune response, Perros and colleagues (2) examined, in cytotoxic T cells, natural killer cells, and natural killer T cells, the expression of granulysin (GNLY), an effector protein regulating these cell populations, both in peripheral blood mononuclear cells and explanted lungs from patients with PAH and PVOD. Using a methylation approach, the authors demonstrated that GNLY demethylation is significantly decreased in genomic DNA from the lungs of patients with PVOD as compared with patients with PAH and control individuals. Also, although a decrease in cytotoxic cells was observed in both PAH and PVOD, GNLY-containing cells in these cytotoxic populations were decreased preferentially in patients with PVOD compared with patients with PAH and control subjects. This contrasted with an increase in serum GNLY concentrations in both PAH and PVOD. Aside from supporting a role for immune dysregulation and loss of cytolytic function in the pathogenesis of PAH and PVOD, this study demonstrates differential epigenetic regulation of GNLY between PVOD and PAH, and might offer a new biological tool (e.g., flow cytometric analysis of GNLY-expressing cytolytic cells) to clinically distinguish these two diseases, an important step considering the significantly poorer prognosis of the former. Finally, whether low expression of GNLY in natural killer cells correlates with more severe forms of pulmonary vascular disease (e.g., PVOD), like it does in cancer progression, is intriguing and remains to be tested (2).

Also strongly in favor of a role for inflammation in the pathogenesis of PH is the finding by Colvin and colleagues of well-organized and exuberant bronchus-associated lymphoid tissue, composed of inflammatory cells such as $CD3^+$ T cells, in animal models of PH (3). These cells appear to produce autoantibodies that, when

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passively transferred into other rats, cause pulmonary vascular remodeling and PH. Various strategies aimed at diminishing lymphoid tissue and the resulting production of autoantibodies reversed PH in this model (3); thus targeting bronchus-associated lymphoid tissue may offer novel therapeutic strategies.

Nitric oxide (NO) signaling appears to play important roles in many forms of PH, ranging from neonatal (4) to diverse adult forms. However, the extent to which dysfunctional NO signaling is responsible for the spectrum of PH is not entirely clear. A review of this topic in the *Journal* concluded that corrections in the deficiency of NO synthesis, or enhancement of its downstream signaling targets such as cGMP and protein kinase G, warrant further study in the treatment of PAH (5). Furthermore, preventing the generation of reactive oxygen species (ROS) by damaged endothelial NO synthase, and limiting the generation of reactive species such as superoxide that can interfere with NO signaling, warrants further study (5).

The shift toward an apoptosis-resistant, proliferative phenotype in pulmonary vascular cells from patients with PAH is retained even after the cells derived from patients are maintained in tissue culture and studied *in vitro*. However, these cells retain the ability to revert to a normal phenotype in response to extracellular cues, which raises the possibility that a similar shift could be induced *in vivo*. Xiao and colleagues studied the role of indoleamine-2,3-dioxygnease (IDO), an enzyme that degrades tryptophan in the cell and shares functional overlap with NO synthase in terms of its ability to promote vasorelaxation through the activation of cGMP-dependent activation of protein kinase G (6). They found that IDO expression was high in lung endothelial cells, and that mice lacking the gene exhibited exaggerated experimental PH. By contrast, augmented pulmonary endothelial expression halted the development of PH and the associated vascular remodeling and right ventricular (RV) hypertrophy in experimental models of PAH. The suppression of proliferation in the pulmonary vascular cells induced by IDO was attributed to enhanced binding of myocardin to specific DNA sequences located in the promoter regions of genes involved in the regulation of smooth muscle differentiation. These results suggest that IDO warrants further study as a potential therapeutic target for the treatment of PH in humans.

On Hypoxia, Oxygen Sensing, and Cellular Respiration

Chronic hypoxia triggers remodeling of the pulmonary arteries characterized by pulmonary arterial smooth muscle proliferation and an increase in wall thickness. The remodeling process begins when oxygen sensors in vascular cells detect a decrease in P_{O_2}, and subsequently activate a signaling system that leads to acute constriction of pulmonary arteries. This hypoxia-induced pulmonary vasoconstrictor response was identified more than 65 years ago, but the underlying mechanism of oxygen sensing was not known. Interestingly, Waypa and colleagues reported that mitochondria function as the site of oxygen sensing underlying this response (7). During hypoxia, mitochondria in vascular cells release superoxide from complex III to the intermembrane space, where it is converted to hydrogen peroxide (H_2O_2) by superoxide dismutase. The H_2O_2 then enters the cytosol, where it activates multiple responses contributing to smooth muscle constriction. The authors demonstrated that the acute production of ROS during hypoxia was abolished in pulmonary artery smooth muscle cells by genetic deletion of the Rieske iron-sulfur protein subunit of complex III. When this gene was deleted in the smooth muscle cells of adult mice, using a *loxP*–Cre system, the *in vivo* hypoxic pulmonary vasoconstrictor response was attenuated. These findings implicate subcellular compartment-specific increases in ROS generation in the acute response to hypoxia, although they do not tell us whether the long-term vascular remodeling during chronic hypoxia is mediated by the same O_2-sensing mechanism (8).

Other evidence implicating mitochondria in PH was provided by Ryan and colleagues, who reported that, in rodent models and in human PH samples, the mitochondria were fragmented (9). This was associated with a decrease in the expression of mitofusin-2 (MFN2), a molecular regulator that promotes the fusion of mitochondria into long tubular structures, and PGC1α, a transcriptional activator of mitochondrial biogenesis. Adenoviral overexpression of MFN2 increased mitochondrial fusion, decreased proliferation, lessened the severity of PH, and improved exercise capacity in the rodent model. These results suggest that decreases in MFN2 and PGC1α contribute to pulmonary vascular remodeling and to an imbalance between apoptosis and proliferation in the pulmonary vasculature. Although the basis for these changes in expression was not explored, in light of the work by Waypa and colleagues (7) it is interesting to speculate that increases in vascular ROS signaling, which have been implicated in the development of PAH, could be responsible for altering the expression of MFN2 and PGC1α, possibly through the activation of hypoxia-inducible factors (HIF-1 and HIF-2).

HIFs have previously been shown to contribute to the remodeling response in the pulmonary circulation in response to alveolar hypoxia (10), and can potentially contribute to the altered regulation of hundreds of genes in diverse cell types. One mechanism by which HIFs could contribute to the vascular remodeling in PH is through the altered expression of membrane ion channels that contribute to the regulation of smooth muscle calcium signaling and proliferation. In that regard, Malczyk and colleagues noted that the classical transient receptor potential channel (TRPC) proteins TRPC1 and TRPC6 are expressed in precapillary pulmonary artery smooth muscle cells (11). These nonselective cation channels could contribute to PH by facilitating Ca^{2+} entry, or by allowing Na^+ entry and thereby inducing Ca^{2+} entry through voltage-dependent channels. They found that TRPC1 mRNA expression was increased by hypoxia in cultured murine pulmonary artery smooth muscle cells, and that cells lacking TRPC1 showed an attenuated proliferation response under hypoxia. Importantly, mice lacking TRPC1 ($TRPC1^{-/-}$) did not develop PH during chronic hypoxia and exhibited less vascular remodeling but a similar degree of RV hypertrophy compared with wild-type mice. These findings implicate TRPC1 as an important contributor to the pulmonary vascular remodeling and the development of PH in response to chronic hypoxia.

Novel Genetic Mutations in PAH

Although mutations in the bone morphogenetic protein receptor-2 gene (*BMPR2*) are present in about 80% of patients with familial PAH and 15% of

patients with idiopathic PAH (IPAH), a search for other genetic mutations is underway. Ma and colleagues (12) identified novel mutations in *KCNK3* (the gene encoding the potassium channel subfamily K, member 3) in a family in which several members were afflicted with PAH without identifiable mutations in genes known to be associated with the disease, such as *BMPR2*, *ALK1*, *ENG*, *SMAD9*, and *CAV1*. While a heterozygous missense variant was identified as a disease-causing candidate gene in this family, 5 additional heterozygous missense variants were identified in 92 unrelated patients with familial PAH and in an additional 230 patients with IPAH. Using elegant electrophysiological studies, the investigators demonstrated that there was loss of function resulting from all missense mutations observed, loss that could be rescued by the use of a pharmacological phospholipase inhibitor (12). KCNK3, which contains four transmembrane domains and two pore domains per subunit, is known for being responsive to hypoxia, regulating resting membrane potential and pulmonary vascular tone, and inhibiting apoptosis. Importantly, KCNK3 can be activated by prostacyclin analogs as well as a protein kinase A activator, making this gene an appealing natural therapeutic target in PAH (12).

More recently, using whole exome sequencing, two independent groups (13, 14) discovered mutations in two diseases that are closely related and can manifest as PAH, pulmonary veno-occlusive disease (PVOD), and pulmonary capillary hemangiomatosis (PCH). PVOD is a rare disorder that may be familial, causing intimal cellular proliferation affecting predominantly the pulmonary venules but also extending to the precapillary pulmonary arteries, resulting in severe PAH. On the other hand PCH, also a rare disease often difficult to distinguish clinically from PVOD, is characterized by uncontrolled endothelial cell proliferation and invasion of capillaries into distal pulmonary arteries and veins, alveoli, intralobular septa, and bronchi, resulting in PAH. Both diseases fail to respond adequately to current PAH therapy. In late 2013, Eyries and colleagues described a set of mutations in the gene for eukaryotic translation initiation factor 2α kinase 4 (*EIF2AK4*) in 13 families with PVOD and an additional 20 patients with presumed sporadic PVOD (13). Also initially published online in 2013 was a report by Best and colleagues describing mutations in *EIF2AK4* in a family with PCH (transmitted as an autosomal recessive pattern) and in two other patients with sporadic PCH (14). It is remarkable that two diseases, with significant clinical overlap (including PAH) but somewhat different histological characteristics, might share a common link with *EIF2AK4*, raising the possibility that these two entities are part of the same spectrum of disease (15). This brings the total list of genes with mutations associated with PAH to seven at the time of this writing.

Preclinical Studies of Potential Therapeutic Benefits

Improving the overall function of genes (e.g., *BMPR2*) containing mutations implicated in the pathogenesis of pulmonary vascular remodeling is an attractive therapeutic strategy based on the notion that nonaffected family members who are carriers of these mutations have higher levels of BMPR2 expression. With this concept in mind, Spiekerkoetter and colleagues (16), using a transcriptional high-throughput luciferase reporter assay to screen 3,756 FDA-approved drugs and other bioactive compounds for their capacity to induce BMPR2 signaling, identified FK506 (tacrolimus) as one of the strongest activators of BMPR2 signaling. In this study, the investigators demonstrated that FK506 interacts with FK-binding protein-12 (FKBP12), a repressor of BMP signaling. FKBP12 inhibits all three forms of the BMPR type 1 receptors (activin receptor-like kinase 1 [ALK1], ALK2, and ALK3). When activated, these receptors trigger downstream signaling through SMAD1/5, mitogen-activated protein kinase, and ID1 gene regulation. Furthermore, low-dose FK506 treatment reversed abnormal BMPR2 signaling in pulmonary endothelial cells (ECs) obtained from patients with IPAH, prevented chronic hypoxic PH in mice with conditional BMPR2 deletion in ECs, and partially reversed pulmonary vascular remodeling and hemodynamic changes in two other rat models of PH (i.e., monocrotaline and a combination of vascular endothelial growth factor receptor blockade with hypoxia). Importantly, FK506 appeared superior to other similar immunosuppressant agents (e.g., rapamycin, known to interact with the immunophilin FKBP12) in potentiating BMPR signaling because of its ability to also inhibit calcineurin. The effect of FK506 was potentially related to restoration of apelin (a proangiogenic and endothelial nitric oxide synthase inducer pSMAD-independent target of BMP signaling) expression. This approach using high-throughput screening to identify new compounds for PAH treatment based on a mechanistic approach is highly original. Also noteworthy in this study is the fact that the *in vivo* preclinical data were obtained with low-dose FK506 (which did not induce systemic hypertension as previously reported with a higher dose), making this FDA–approved drug a high contender for further clinical studies in PAH (16).

The proliferative phenotype of pulmonary vascular cells in some patients with PAH may involve dysregulated growth factor signaling. The membrane receptors for platelet-derived growth factor, epidermal growth factor, fibroblast growth factor, and other mitogens trigger cell proliferation via a common mechanism involving increased tyrosine kinase (TK) activity when bound to their ligands. Dysfunctional growth factor signaling develops in many tumors, and drugs that inhibit TK activity are used as anticancer therapeutics. Imatinib, a TK inhibitor, also has been shown to improve hemodynamics in a subset of patients with PH. Ciuclan and colleagues studied the effects of imatinib in a rodent model, and found that it ameliorated the development of PH (17). A secondary effect of the drug was that it decreased expression of tryptophan hydroxylase 1 (Tph1), the rate-limiting enzyme involved in 5-hydroxytryptamine (5-HT, serotonin) synthesis, while it lowered 5-HT levels. The importance of Tph1 down-regulation in the salutary effects on PH was demonstrated by showing that knockout mice lacking Tph1 did not exhibit the same degree of protection by imatinib that was seen in wild-type mice. These findings indicate that imatinib may exert some of its protective effects by decreasing 5-HT levels, in addition to its effects on growth factor signaling. Although pharmacological inhibition of the 5-HT pathway represents a potentially useful therapeutic benefit, the currently available 5-HT receptor antagonists and transporter inhibitors suffer from side effects and limited efficacy. The ability of imatinib to inhibit both the TK and 5-HT pathways is therefore an exciting discovery (18).

Angiotensin-converting enzyme-2 (ACE2) is a monocarboxypeptidase that

metabolizes angiotensin II to angiotensin-(1–7). While the classical ACE–angiotensin II–angiotensin 1 receptor axis of the renin–angiotensin system promotes vasoconstriction and PH, the ACE2–angiotensin-(1–7)–Mas axis suppresses vasoconstriction and protects against PH, making it an attractive candidate for pharmacological targeting in the treatment of PAH. Shenoy and colleagues reported that an antitrypanosomal drug, diminazene, exerts an off-target effect resulting in the activation of ACE2 (19). In rodent models of PH induced by monocrotaline, hypoxia, or bleomycin, they found that diminazene treatment prevented the development of PH while it also decreased inflammatory cytokines and enhanced cardiac function in an ACE2-dependent manner. At least some of the beneficial effects may have been the result of an improvement in the migratory capacity of bone marrow–derived progenitor cells (19). Although these preclinical results are intriguing, further studies are required to determine more clearly the mechanism of action and to assess whether multiple models of PH can effectively be treated with this drug (20). Importantly, studies to determine the safety in humans will need to be performed, even though the drug has been used previously in humans for the treatment of tropical parasitic disease.

On Causes of Death and New Therapies in PAH

Improved biomarkers are needed clinically to potentially provide useful information on the severity, prognosis, and response to treatment in patients with PAH. MicroRNAs (miRs) play important roles in the regulation of gene expression, both in health and disease. Rhodes and colleagues performed a microarray screen to detect plasma RNA in eight patients with PAH and in normal control subjects (21). Differences in 58 miRs were detected, and miR-158 was identified as the most suppressed in the patient samples. Moreover, the decrease in miR-158 was associated with 2-year survival in patients with PAH, along with other indices of disease severity such as the 6-minute walk distance. These initial findings were replicated in a second independent cohort, identifying the decrease in miR-158 as an independent negative predictor of survival in this disease.

An interesting development in clinical trials in PAH has been a shift away from the 6-minute walk distance as an end point to a more composite end point that includes death as discussed below regarding the SERAPHIN (Study with an Endothelin Receptor Antagonist in Pulmonary Arterial Hypertension) study. Regarding death, the report by Tonelli and colleagues on the causes and circumstances of death in PAH (22) is noteworthy. It has long been recognized that right ventricular failure is the leading cause of death in this syndrome; however, no study has specifically tackled this problem. Although autopsies were not performed in their study, the authors examined the direct causes of death and circumstances surrounding death in 84 patients with PAH monitored at their institution between 2008 and 2012 by carefully scrutinizing the medical records. They determined that pulmonary hypertension was directly related or contributed to death in 44% of the cases, respectively (total of 88%), with right heart failure/sudden death being the most common occurrence (44%). Several findings concerning the circumstances of death were also interesting. Most patients died in a health care environment (typically the intensive care unit) with more than half of these patients having no advanced health care directives. Fifty percent of patients with PAH as well as 75% of patients who died with right ventricular failure were receiving parenteral prostanoid therapy, which is in sharp contrast with data from the Registry to Evaluate Early and Long-Term PAH Disease Management (REVEAL), in which less than half of the patients with very advanced PAH disease were reported to be receiving such therapy, which suggests gross underuse of a drug generally thought to improve survival. Several conclusions can be drawn from this important study: although survival may be improving in patients with PAH receiving modern therapy, causes of death still overwhelmingly related to PH may be slowly changing. Patients with PAH live longer, have somewhat different demographics, but continue to die seemingly unprepared (22, 23).

A poorly explored factor has been the impact of socioeconomic status on outcomes, particularly death, in patients with PAH. Although low socioeconomic status is known to negatively impact outcomes in cardiovascular and pulmonary diseases, its role in PAH has not been fully explored. Yet this is an important question considering the high cost of PAH drugs and the widening gap between rich and poor in the United States. Interestingly, initial insight into this important question comes from a prospective cohort of Chinese patients with PAH. Using a growing database of Chinese patients with IPAH referred to their national center, Wu and colleagues demonstrated that, after adjustment for clinical features, hemodynamics, and type of PAH treatment, the hazard ratios for death were significantly increased in the lower and middle tertile of socioeconomic status (2.98 and 1.80, respectively) compared with the upper tertile (24). Similar studies would be welcome in the United States, particularly in view of the ongoing raging debate on health care issues and coverage.

Although phosphodiesterase inhibitors (PDE-Is) such as sildenafil have now been used extensively in the adult and pediatric population with PAH and are recognized to be safe long-term at least in adults, a FDA strong warning recommended against the use of sildenafil in children between the ages of 1 and 17. This was based on an apparent increase in mortality in patients treated at high dosage in a single trial (25) and subsequent long-term follow-up data obtained from this trial. This somewhat unexpected warning motivated Abman and colleagues from the Pediatric Pulmonary Hypertension Network (PPHNet) to strongly react and propose several recommendations including the avoidance of high doses of sildenafil and abrupt discontinuation of the drug in children (26). Since then, the FDA issued a clarification stating that the prior recommendation was not intended to suggest that sildenafil should never be used in children, and that situations may exist where the benefit–risk balance may be acceptable in individual children, especially when used during close monitoring. Clearly more is to be learned about the safety and dosage of PDE-I in children, and particularly in infants.

Other drugs have been added to the rapidly growing armamentarium of PAH therapies. Macitentan is a novel dual endothelin receptor antagonist that was approved by the FDA on the basis of a clinical trial that used as a novel primary end point the time from initiation of treatment to the first occurrence of

a composite end point (death, atrial septostomy, lung transplantation, initiation of prostanoids, or worsening PAH) (27). Macitentan (added to background PAH therapy or placebo) significantly reduced morbidity and mortality among patients with PAH in this first of its kind event-driven study (27). Strengths of this clinical trial include the large number of patients and the novelty of the primary composite end point used, which is more relevant than the 6-minute walk distance used in other studies. This could establish a precedent as the primary end point for future PAH clinical trials. However, it should be emphasized that although macitentan therapy improved the composite end point as defined previously, it did not improve overall mortality in these patients as might be inferred by the title (27).

Riociguat, another drug approved by the FDA, is a stimulator of soluble guanylate cyclase (sGC), a key enzyme in the nitric oxide signaling pathway, and constitutes the first drug of a novel class of sGC stimulators in the treatment of PAH. Two phase III clinical trials demonstrated that riociguat significantly improved exercise capacity in patients with PAH and chronic thromboembolic pulmonary hypertension (28, 29).

While new pathways are being targeted in animal models of PH, therapies that have now proven useful for many decades for the treatment of left ventricular failure are, after initial hesitation, being reconsidered for the treatment of PAH and RV failure. Among these are drugs targeting the neurohormonal systems including the sympathetic nervous system (SNS) and the renin–angiotensin–aldosterone system (RAAS). In a concise yet carefully crafted perspective, de Man and colleagues reviewed the rationale for targeting these systems (30). Alterations in SNS activation (e.g., increased sympathetic nerve activity and changes in the RV expression of adrenergic receptors) have long been recognized in PAH; more recently, however, the authors have demonstrated evidence of RAAS activation (as determined by increased plasma levels of renin and angiotensin I and II). Activation of these two systems are of interest as they are associated with poor outcomes in PAH, although it remains to be determined whether they are a cause or consequence (e.g., in response to RV wall stress) of PAH. However, these observations have renewed interest in the use of β-blocker therapy, ACE inhibitors, and AT1-receptor blockers, as well aldosterone and angiotensin-converting enzyme antagonists, all obviously used successfully in left heart failure and in several animal models of PH. Also of growing interest is the use of nonpharmacological interventions to decrease SNS activity, such as performance of atrial septostomy or simple exercise training, while clinical trials for safety and efficacy are required to test the effect of pharmacological interventions (such as blockers of SNS or RAAS) (30).

Thromboembolic Disease

Thromboembolic disease continues to carry great morbidity and mortality, and often remains difficult to diagnose in a timely fashion because of its nonspecific clinical presentation. In that regard, den Exter and colleagues sought to assess the impact of presentation delay on the diagnostic management and clinical outcomes in patients with suspected pulmonary embolism (PE) (31). The authors used combined data from two large prospective outcome trials in the Netherlands dedicated to studying the diagnostic management of patients with suspected PE. They compared, among 4,044 consecutive patients with suspected PE, the clinical characteristics and outcomes of patients presenting more than 7 days from the onset of symptoms with those from patients presenting within 7 days of their symptoms. The authors focused mainly on the safety of excluding PE based on clinical decision (using the Wells score) combined with D-dimer testing. Delayed presentation was common (18.4% of the whole cohort). However, the failure of an unlikely diagnosis using this algorithm was extremely low (0.5%) in both groups. On the other hand, the sensitivity of D-dimer was high in both groups (99 and 98%, respectively). Interestingly, patients who had a diagnostic delay were more likely to have centrally located PE (41 vs. 26%). Finally, the cumulative rates of recurrent venous thromboembolism and mortality were similar in the two groups. This study suggests that (*1*) a delay in diagnosis is common; (*2*) using a simple algorithm for excluding the diagnosis is valid even in delayed diagnosis (including delayed performance of D-dimer measurement); and (*3*) patients with delayed diagnosis are more likely to have centrally located clots. In addition, if they survive their thromboembolic disease, they are likely to do just as well as patients diagnosed promptly within 7 days of presentation (31).

Another large study from Europe (Denmark in this case) explored the use of elevated plasma fibrinogen for the diagnosis of deep venous thromboembolism (DVT) and PE in more than 77,000 individuals, of whom 1,679 were diagnosed with DVT alone, 1,119 with PE, and 272 with both diagnoses. Elevated plasma levels were associated with an increased risk of PE in combination with DVT but not with DVT alone (32). Because plasma fibrinogen levels influence the structural properties of a developing fibrin gel in DVT, the authors hypothesize that elevated levels may influence expansion or fracture of the DVT thrombus rather than efficient lysis. Conditions that were associated with elevated plasma fibrinogen levels in this study included specific polymorphisms (homozygotes of the mutation vs. noncarriers) in the β-fibrinogen gene, with a further increase in overweight patients, smokers, and particularly patients with elevated C-reactive protein levels. Surprisingly, however, these genetic variants were not associated with an increased risk of DVT or PE (32).

Conclusions

As reflected by the breadth of papers published in the *Journal* and elsewhere, there has been tremendous progress in all fields of pulmonary vascular disease including diagnosis, understanding mechanisms of disease, use of novel therapeutic targets, and refining our methods to quantitatively assess remodeling of the pulmonary vasculature, for example, by the use of computed tomographic scanning of the chest in diseases that can be complicated by vascular remodeling such as COPD (33). The list of gene mutations associated with PAH continues to grow, with two additional genes since the last update on pulmonary vascular disease. It is expected that this year will continue to attract young scientists eager to contribute to the remarkable advancement in this field. Our hope is to continue to be richly represented in articles published in the *Journal*. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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