(23, 24), thus allowing vitamin D deficiency to affect the developing lung and the subsequent risk of asthma via multiple mechanisms.

The public health importance of the hypothesis that vitamin D is critically related to asthma is fueled by the knowledge that currently over 90% of African-American pregnant women and over 60% of white pregnant women are vitamin D–deficient or –insufficient (25, 26). This is not true of other major hormonal moderators of lung development such as vitamin A. Clinical trials of vitamin D to prevent asthma are currently underway in both the United States (Vitamin D Antenatal Asthma Reduction Trial [VDAART], U01 HL091528) and in Denmark (ClinicalTrials.gov: NCT00856947). These studies will test only part of vitamin D's influence on asthma risk, namely the fetal origins effect. What will not be tested in these trials is the relative importance of postnatal vitamin D sufficiency versus *in utero* sufficiency in asthma prevention. It is quite likely that both pre- and postnatal sufficiency will be required to maximally lower risk. There are still many studies that need to be performed on vitamin D and its protean health effects—for example, its effect on pre-eclampsia, preterm birth generally, and hence on Respiratory Distress Syndrome of the newborn and subsequent bronchopulmonary dysplasia. These initial trials should at least begin to provide the clinical basis for determining whether there is a role of vitamin D as a preventative for asthma, but there is much more work to be done.

Author Disclosure: S.T.W. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. A.A.L. has received royalties from UpToDate, Inc.

SCOTT T. WEISS, M.D., M.S.
AUGUSTO A. LITONJUA, M.D., M.P.H.
*Brigham and Women's Hospital
Harvard Medical School
Boston, Massachusetts*

References

- Camargo CA, Jr, Rifas-Shiman SL, Litonjua AA, Rich-Edwards JW, Weiss ST, Gold DR, Kleinman K, Gillman MW. Maternal intake of vitamin D during pregnancy and risk of recurrent wheeze in children at age 3 years. *Am J Clin Nutr* 2007;85:788–795.
- Devereux G, Litonjua AA, Turner S, Rich-Edwards JW, Weiss ST, Gold DR, Kleinman K, Gillman MW. Maternal vitamin D intake during pregnancy and early childhood wheezing. *Am J Clin Nutr* 2007;85:853–859.
- Miyake Y, Sasaki S, Tanaka K, Hirota Y. Maternal B vitamin intake during pregnancy and wheeze and eczema in Japanese infants aged 16–24 months: The Osaka Maternal and Child Health Study. *Pediatr Allergy Immunol* 2011;22:69–74.
- Erkkola M, Kaila M, Nwaru BI, Kronberg-Kippilä C, Ahonen S, Nevalainen J, Veijola R, Pekkanen J, Ilonen J, Simell O, *et al*. Maternal vitamin D intake during pregnancy is inversely associated with asthma and allergic rhinitis in 5-year old children. *Clin Exp Allergy* 2009;39:875–882.
- Brehm JM, Celedon JC, Soto-Quiros ME, Avila L, Hunninghake GM, Forno E, Laskey D, Sylvia JS, Hollis BW, Weiss ST, *et al*. Serum vitamin D levels and markers of severity of childhood asthma in Costa Rica. *Am J Respir Crit Care Med* 2009;179:765–771.
- Brehm JM, Schuemann B, Fuhlbrigge AL, Hollis BW, Strunk RC, Zeiger RS, Weiss ST, Litonjua AA. Serum vitamin D levels and

- severe asthma exacerbations in the Childhood Asthma Management Program study. *J Allergy Clin Immunol* 2010;126:52–8.e5.
- Sutherland ER, Goleva E, Jackson L, Stevens A, Leung D. Vitamin D levels, lung function, and steroid response in adult asthma. *Am J Respir Crit Care Med* 2010;181:699–704.
- Black PN, Scragg R. Relationship between serum 25-hydroxyvitamin D and pulmonary function in the third national health and nutrition examination survey. *Chest* 2005;128:3781–3783.
- Zosky GR, Berry L, Elliot J, James AL, Gorman S, Hart PH. Vitamin D causes deficits in lung function and alters lung structure. *Am J Respir Crit Care Med* 2011;183:1336–1343.
- Barker D, Eriksson J, Forsen T, Osmond C. Fetal origins of adult disease: strength of effects and biological basis. *Int J Epidemiol* 2002;31:1235–1239.
- Yunginger JW, Reed CE, O'Connell EJ, Melton LJ III, O'Fallon WM, Silverstein MD. A community-based study of the epidemiology of asthma: incidence rates, 1964–1983. *Am Rev Respir Dis* 1992;146:888–894.
- Burri PH. Fetal and postnatal development of the lung. *Annu Rev Physiol* 1984;46:617–628.
- Phokela SS, Peleg S, Moya FR, Alcorn JL. Regulation of human pulmonary surfactant protein gene expression by 1 α ,25-dihydroxyvitamin D₃. *Am J Physiol Lung Cell Mol Physiol* 2005;289:L617–L626.
- Glasgow JF, Thomas PS. Rachitic respiratory distress in small preterm infants. *Arch Dis Child* 1977;52:268–273.
- Rehan VK, Torday JS, Peleg S, Gennaro L, Vouros P, Padbury J, Rao DS, Reddy GS. 1 α ,25-dihydroxy-3- ϵ -pi-vitamin D₃, a natural metabolite of 1 α ,25-dihydroxy vitamin D₃: production and biological activity studies in pulmonary alveolar type II cells. *Mol Genet Metab* 2002;76:46–56.
- Gaultier C, Harf A, Balmain N, Cuisinier-Gleizes P, Mathieu H. Lung mechanics in rachitic rats. *Am Rev Respir Dis* 1984;130:1108–1110.
- Lunghi B, Meacci E, Stio M, Celli A, Bruni P, Nassi P, Treves C. 1,25-dihydroxyvitamin D₃ inhibits proliferation of IMR-90 human fibroblasts and stimulates pyruvate kinase activity in confluent-phase cells. *Mol Cell Endocrinol* 1995;115:141–148.
- Stio M, Celli A, Lunghi B, Raugi G, Modesti A, Treves C. Vitamin D receptor in IMR-90 human fibroblasts and antiproliferative effect of 1,25-dihydroxyvitamin D₃. *Biochem Mol Biol Int* 1997;43:1173–1181.
- Sakurai R, Shin E, Fonseca S, *et al*. 1 α , 25(OH)₂D₃ and its 3- ϵ -epimer promote rat lung alveolar epithelial-mesenchymal interactions and inhibit lipofibroblast apoptosis. *Am J Physiol Lung Cell Mol Physiol* 2009;297:L496–L505.
- Kho A, Bhattacharya S, Tantisira K, Carey VJ, Gaedigk R, Leeder JS, Kohane IS, Weiss ST, Mariani TJ. Transcriptomic analysis of human lung development. *Am J Respir Crit Care Med* 2010;181:54–63.
- Bossé Y, Lemire M, Poon AH, Daley D, He JQ, Sandford A, White JH, James AL, Musk AW, Palmer LJ, *et al*. Asthma and genes encoding components of the vitamin D pathway. *Respir Res* 2009;10:98.
- Damera G, Fogle H, Lim P, Goncharova EA, Zhao H, Banerjee A, Tliba O, Krymskaya VP, Panettieri RA Jr. Vitamin D inhibits growth of human airway smooth muscle cells through growth factor-induced phosphorylation of retinoblastoma protein and checkpoint kinase 1. *Br J Pharmacol* 2009;158:1429–1441.
- Chambers E, Hawrylowicz CM. The impact of vitamin D on regulatory T cells. *Curr Allergy Asthma Rep* 2011;11:29–36.
- Lange N, Litonjua AA, Hawrylowicz CM, Weiss S. Vitamin D, the immune system and asthma. *Expert Rev Clin Immunol* 2009;5:693–702.
- Hollis BW, Wagner CL. Assessment of dietary vitamin D requirements during pregnancy and lactation. *Am J Clin Nutr* 2004;79:717–726.
- Hollis BW, Wagner CL. Nutritional vitamin D status during pregnancy: reasons for concern. *CMAJ* 2006;174:1287–1290.

DOI: 10.1164/rccm.201101-0160ED

Focusing on the Flood Targeting Functional Polymorphisms in ALI Permeability Pathways

Despite considerable efforts, disappointingly few significant advances have been achieved in the management of patients

with devastating acute lung injury (ALI) with the pace for novel ALI insights, biomarkers, and interventions excruciatingly slow. Over the past decade, only alterations in ventilator management have served as an impactful novel modality. In this light, the post-genome era held promise that application of high-

Supported by National Heart, Lung, and Blood Institute/National Institutes of Health grants HL094394 and HL058064.

throughput genomic and genetic technologies might accelerate ALI discovery and advantage the personalization of ALI medical care. As only a minority of patients exposed to ALI-predisposing insults manifest ALI, substantial support was generated for individual genetic variation as a key contributor to ALI susceptibility. However, in contrast to the approximately 200 complex disorders with published genome-wide association studies, large-scale genotyping has not yet been conducted in either ALI cohorts or in cohorts afflicted with the major ALI-inciting causes such as sepsis and trauma. Consequently, ALI genetic studies have employed a candidate gene approach, often leveraging genomic-intensive strategies, with case-control studies surprisingly effective in identifying single-nucleotide polymorphisms (SNPs), which confer differential risk of developing ALI or influence ALI mortality (1–3), several of which have been validated in replicate cohorts (4, 5). Unfortunately, many candidate gene studies have been difficult to replicate, either due to limited sample sizes, population stratification, variability of the control population, or heterogeneity of the ALI phenotype. Thus, while there are multiple lines of evidence supporting genetic susceptibility to ALI, many ALI candidate genes failed replication in other ALI cohorts. Furthermore, few genetic studies have been conducted in multiple ALI ethnicities, and large-scale ALI genotyping has not yet been reported.

A recent alternate approach to identification of validated ALI candidate genes has focused on genes centrally involved in biological pathways relevant to the complex pathophysiology of ALI. This strategy has highlighted genes involved in epithelial and vascular barrier function, paralleling growing interest in vascular homeostatic gene products as novel ALI biomarkers. Potential ALI vascular biomarkers and targets for genetic variation include inflammatory factors (IL-1 β , IL-6, IL-8, TNF- α), coagulation factors (protein C, thrombomodulin), and endothelial cell-derived gene products such as von Willebrand Factor, intercellular adhesion molecule-1, and E- or P-selectin. As marked disruption of vascular integrity, resulting in profound lung permeability and alveolar flooding, is a cardinal feature of the inflamed ALI lung, this focus would appear to be well justified, especially as ALI severity and outcome are dependent on the magnitude of alveolar epithelial and/or vascular injuries. The list of vascular-related ALI genes has grown to include angiogenic factors such as vascular endothelial growth factor (VEGF), hepatocyte growth factor, and sphingosine 1-phosphate, all agonists that influence vascular integrity (6–8), as well as cytoskeletal signaling effectors (2, 4). For example, through the potent angiogenic factor, VEGF, previously known as vascular permeability factor, lung vascular barrier function is compromised and is an important mechanism responsible for increased vascular permeability, pulmonary edema, and leukocyte migration into lung tissues resulting in persistent inflammation. In addition, angiogenic mediators such as VEGF, epidermal growth factor, and matrix metalloproteinases have been implicated in ALI pathogenesis and individually associated with ALI risk (9, 10).

In this issue of the *Journal*, Meyer and coworkers (pp. 1344–1353) have addressed an important gap in ALI genetic studies by genotyping a cohort of critically ill trauma subjects of African and European descent utilizing a cardiopulmonary disease-centric 50K SNP array (11). These studies identified a region in the angiogenic factor gene, angiopoietin-2 (*ANGPT2*), to be associated with increased risk for trauma-associated ALI, results validated in a multicenter European American trauma-associated ALI case-control population. While this report confirms prior report of *ANGPT2* genetic variants associated with the development of ALI (12), the high merit of this strategy of SNP discovery in a cohort of African descent is supported by the rich

genetic diversity and small block size; and, despite the unconventional approach of discovering in a small AA population and replicating in EA, it points to an association that is robust across ethnicities. While the African discovery cohort is relatively small (~ 200 cases), this is comparable to the largest African descent cohorts in ALI (13), highlighting the need for accumulation of African descent-rich ALI cohorts.

Angiopoietin-2 (ANG2), a naturally occurring antagonist for angiopoietin-1 (ANG1), an angiogenic factor essential for normal vascular development, is well recognized to impact lung permeability in experimental models of lung injury. ANG2 has been implicated in pulmonary vascular leak syndromes including ALI and sepsis in both animal and human studies, and elevated ANG2 levels are detected in the blood and bronchoalveolar lavage fluid of patients with ALI and conferred risk of ALI in trauma patients (14). Importantly, in the current study, Meyer and colleagues linked the *ANGPT2* SNP with increased levels of a variant ANG2 isoform in plasma, suggesting that the risk polymorphism tags a splice site enhancer or novel splice site. Resequencing identified predicted novel splice sites in linkage disequilibrium with the *ANGPT2* SNP and immunoblots showed higher proportion of variant ANG2 isoform associated with this SNP. While the observed ANG2 isoforms remain to be confirmed as ANG2 A and C, these results show the potential relevance of circulating ANG2 and may shift attention to the effect of variation in the coiled-coil region of ANG2 on vascular permeability regulation.

In summary, the study of the genetic contributions to ALI pathogenesis, severity, and response to therapy remains a nascent, albeit exciting field that holds great promise, with defining functional relationships of genetic variants remaining a challenge. Complementing the utility of combining advanced bioinformatic techniques and multi-species gene expression profiling as a way to broaden the net for ALI-related genes, interrogation of biologically important pathways, such as permeability regulation, also serves to provide high-yield strategy for identification of novel genetic targets and biomarkers. This pathway approach is the ideal complement to more traditional, hypothesis-based testing of candidate genes, and when linked to genome-wide approaches, is anticipated to fuel additional analysis of new candidates.

Author Disclosure: The author does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

JOE G. N. GARCIA, M.D.
University of Illinois at Chicago
Chicago, Illinois

References

1. Ye SQ, Simon BA, Maloney JP, Zambelli-Weiner A, Gao L, Grant A, Easley RB, McVerry BJ, Tudor RM, Standiford T, *et al*. Pre-B-cell colony-enhancing factor as a potential novel biomarker in acute lung injury. *Am J Respir Crit Care Med* 2005;171:361–370.
2. Gao L, Grant A, Halder I, Brower R, Sevransky J, Maloney JP, Moss M, Shanholtz C, Yates CR, Meduri GU, *et al*. Novel polymorphisms in the myosin light chain kinase gene confer risk for acute lung injury. *Am J Respir Cell Mol Biol* 2006;34:487–495.
3. Wurfel MM, Gordon AC, Holden TD, Radella F, Strout J, Kajikawa O, Ruzinski JT, Rona G, Black RA, Stratton S, *et al*. Toll-like receptor 1 polymorphisms affect innate immune responses and outcomes in sepsis. *Am J Respir Crit Care Med* 2008;178:710–720.
4. Christie JD, Ma SF, Aplenc R, Li M, Lanken PN, Shah CV, Fuchs B, Albelda SM, Flores C, Garcia JG. Variation in the myosin light chain kinase gene is associated with development of acute lung injury after major trauma. *Crit Care Med* 2008;36:2794–2800.
5. Bajwa EK, Yu CJ, Gong MN, Thompson BT, Christiani DC. Pre-B-cell colony-enhancing factor gene polymorphisms and risk of acute respiratory distress syndrome. *Crit Care Med* 2007;35:1424–1425.

6. Liu F, Schaphorst KL, Verin AD, Jacobs KN, Burkova A, Day RM, Garcia JGN. Hepatocyte growth factor enhances cytoskeletal rearrangement and endothelial cell barrier function and cortical cytoskeletal rearrangement: potential role of glycogen synthase kinase 3 β . *FASEB J* 2002;16:950–962.
7. Garcia JGN, Liu F, Wang P, Verin AD, Dechert MA, Bamburg J, Gerthoffer WT, English DK. Sphingosine 1-phosphate promotes vascular endothelial cell barrier integrity by Edg receptor-dependent cytoskeletal rearrangement. *J Clin Invest* 2001;108:689–701.
8. Kaner RJ, Ladetto JV, Singh R, Fukuda N, Matthay MA, Crystal RG. Lung overexpression of the vascular endothelial growth factor gene induces pulmonary edema. *Am J Respir Cell Mol Biol* 2000;22:657–664.
9. Sheu CC, Zhai R, Su L, Tejera P, Gong MN, Thompson BT, Chen F, Christiani DC. Sex-specific association of epidermal growth factor gene polymorphisms with acute respiratory distress syndrome. *Eur Respir J*. 33:543–550.
10. Medford AR, Keen LJ, Bidwell JL, Millar AB. Vascular endothelial growth factor gene polymorphism and acute respiratory distress syndrome. *Thorax* 2005;60:244–248.
11. Meyer N, Mingyao L, Rui F, Bradfield J, Gallop R, Bellamy S, Fuchs BD, Lanken PN, Albelda SM, Rushefski M, *et al.* ANGPT2 Genetic Variant Is Associated with Trauma-Associated Acute Lung Injury and Altered Plasma Angiopoietin-2 Isoform Ratio. *Am J Respir Cell Mol Biol* 2011;183:1344–1353.
12. Su L, Zhai R, Sheu CC, Gallagher DC, Gong MN, Tejera P, Thompson BT, Christiani DC. Genetic variants in the angiopoietin-2 gene are associated with increased risk of ARDS. *Intensive Care Med* 2009;35:1024–1030.
13. Gao L, Flores C, Ma SF, Miller EJ, Moitra J, Moreno L, Wadgaonkar R, Simon B, Tudor R, Brower R, *et al.* Role of macrophage migration inhibitory factor (MIF) in acute lung injury (ALI) and sepsis: association of a promoter polymorphism and increased gene expression. *Transl Res* 2007;150:18–29.
14. Orfanos SE, Kotanidou A, Glynos C, Athanasiou C, Tsigkos S, Dimopoulou I, Sotiropoulou C, Zakynthinos S, Armaganidis A, Papapetropoulos A, *et al.* Angiopoietin-2 is increased in severe sepsis: correlation with inflammatory mediators. *Crit Care Med* 2007;35:199–206.

DOI: 10.1164/rccm.201104-0623ED

Straining to Make Mechanical Ventilation Safe and Simple

It is remarkable how many research studies and manuscripts are devoted to the topic of ventilation-induced lung injury (VILI) decades after its initial description (1). This tells us that the topic is important, complex, and on some level still poorly understood. This is not to say that little has been learned during the ensuing four decades. Quite to the contrary, the discovery has widened the horizons of clinicians, physiologists, epidemiologists, engineers, biophysicists, immunologists, geneticists, and molecular biologists, and has fostered fruitful collaborations across seemingly divergent fields of biomedical science. While the motivations and perceptions of important knowledge gaps may vary among these disciplines, to a clinician the all-important question is this: “How do I turn the knobs on the ventilator to avoid this catastrophic iatrogenic complication?” For a while it seemed as if the ARDS-Network study on the efficacy of low-tidal-volume mechanical ventilation (the ARMA trial) had taught clinicians all they needed to know (2). However, uncertainties about “best PEEP” (positive end-expiratory pressure), the appropriate plateau pressure target (Pplat), value of recruitment maneuvers, and the efficacy of closed loop modes and high-frequency oscillation (to name only a few) have kept VILI at the forefront of mechanical ventilation-motivated clinical and translational research.

In this issue of the *Journal*, Protti and colleagues (pp. 1354–1367) report on the susceptibility of healthy porcine lungs to injury caused by mechanical ventilation with large tidal volumes (V_T) and conclude that lung damage only occurs when a strain greater than 1.5 to 2.0 is reached/overcome (3). Strain was defined as the fractional volume change between functional residual capacity (FRC) and the thoracic gas volume at end-inflation. Since none of the animals was ventilated with PEEP, strain equaled V_T /FRC. The nonlinear relationship between strain and subsequent injury suggested a threshold phenomenon with a large range of apparently “safe” V_T values. Injurious strains resulted in end-inspiratory lung volumes that fell above the linear portion of the respiratory system pressure–volume curve and hence were associated with disproportionately large parenchymal stresses. In that respect, the observations are entirely in line with the stress index hypothesis, which states that the preferred volume range over which lungs of patients with respiratory failure should be

ventilated is defined by the linear portion of the respiratory system pressure–volume curve (4, 5). Protti and colleagues’ findings are of particular interest not only because of their clinical implications, but also because the experimental approach was meticulous, the experiments were performed over many hours, and the care of the animals was comparable to that provided to anesthetized humans. It is of note that many animals that ultimately died from “pulmonary stress failure” seemed uninjured for long periods of time. However, once changes in respiratory system impedance became manifest, the animals decompensated rapidly. Consistent with the proposed pathogenesis, during volume preset ventilation the dropout of lung units increases the strain on the remaining open ones, setting in motion a “cascade of falling dominos” that culminates in organ failure.

Since Protti and colleagues place tissue strain at the center of the VILI pathogenesis, it might be useful to consider strain-dependent injury mechanisms and to relate strain to the more familiar ventilator parameters PEEP, V_r , and Pplat. A strain is a normalized measure of deformation representing the displacement between particles in the body relative to a reference length. For the purpose of this discussion one can think of strain as the fractional length change of a lung tissue element relative to its initial length. If the lung were an ideal elastic material, one in which energy is stored without dampening losses, its “injury threshold” would be defined by a single length (or volume) above which the material begins to yield (is permanently deformed). The injury threshold would not be sensitive to the rate of elongation (strain rate), nor would the initial length have any influence on the probability of tissue failure. However, like all other biological materials, the elastic properties of the lung are anything but “ideal,” insofar as global stress and tissue micro-architecture are sensitive to length history and time (6). Consequently, at a given end-inspiratory volume, transpulmonary pressure (a measure of lung parenchymal stress) varies with the rate of lung inflation as well as the initial or starting volume (7, 8). On a smaller scale, yet one that is relevant from a mechanotransduction and hence tissue injury perspective, rate and amplitude of deformation exert profound effects on cytoskeletal fluidity and hence cytoskeletal architecture (9, 10), on the hydration state of ground substance (11, 12), and consequently on a host of enzymes such as metalloproteinases, which are involved in inflammatory signaling and tissue remodeling responses (13).