yet to translate into meaningful clinical effects. Amiloride, the prototypical ENaC inhibitor, increases the ASL height of cultured airway, but several clinical trials have failed to demonstrate appreciable benefit after nebulized administration (14, 15). A long-acting and potent amiloride analog, GS-9411, had initial promise as well, but unfortunately, systemic effects including hyperkalemia limited its potential in early clinical trials (16). An additional long-acting ENaC inhibitor, Parion-1037 (now VX-371), is currently in clinical trials, and the results remain pending. SPX-101 has several distinguishing features that may improve its efficacy compared with amiloride derivatives. First, SPX-101 has a prolonged duration of action that may provide durable ASL expansion, simplify dosing, and prolong the effects of hydration strategies such as nebulized hypertonic saline. Another compelling feature of SPX-101 is that systemic effects of ENaC inhibition, such as hyperkalemia or volume depletion, have not been observed in preclinical animal models. Although Tarran and colleagues have made impressive progress in identifying SPLUNC1 and developing SPX-101, it remains to be seen whether these efforts will translate into clinical benefit, particularly above that seen with hypertonic saline, for individuals with CF. Novel therapeutics such as SPX-101 and the next-generation cystic transmembrane conductance regulator correctors make the CF future bright, and we look forward to these upcoming studies. ■

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The Epigenetics of Intrauterine Smoke Exposure: Can Maternal Vitamin C Supplementation Prevent Neonatal Respiratory Disease?

Intrauterine smoke (IUS) exposure has been associated with a myriad of adverse health outcomes in the newborn, including decrements in lung function that persist into adolescence (1), early-onset wheeze (2), and asthma susceptibility (3). The

public health implications of these transgenerational effects of maternal smoking indicate an urgent need to better understand their biologic underpinnings and to identify early intervention strategies.

Developmental processes are highly regulated by epigenetic mechanisms, and the epigenome is most vulnerable to environmental perturbations during fetal development when the DNA synthesis

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rate is high (4). It has been postulated that epigenetic modifications mediate the adverse developmental consequences associated with maternal smoking during pregnancy and that changes in DNA methylation due to IUS exposure play a critical role in the developmental origin of adult respiratory disease.

IUS exposure has been previously associated with changes in DNA methylation in human fetal lung (5), placenta (5, 6), newborn cord blood (7–11), and the buccal mucosa of children (12). Previous studies have also established that there is a dose–response relationship to the DNA methylation changes identified in the newborn that is dependent on the duration and intensity of the intrauterine exposure and that many of these DNA methylation modifications that result from IUS exposure persist into adolescence (10).

Vitamin C, an essential vitamin in humans, with antioxidant properties, is an important cofactor in DNA methylation. During normal development, fetal vitamin C concentrations in the plasma are higher than maternal levels, suggesting that it may be of particular importance to the developing fetus (13). Cigarette smoke exposure is known to decrease plasma vitamin C levels (14), which are commonly deficient in pregnant smokers (15). Animal models have demonstrated that vitamin C supplementation during pregnancy ameliorates the effect of prenatal nicotine exposure on pulmonary function in newborns (16). Furthermore, a recent small randomized controlled trial demonstrated that vitamin C supplementation in pregnant smokers was associated with improved measures of pulmonary function in the newborn and in decreased wheeze at 1 year (15). However, the impact of vitamin C supplementation on the DNA methylation patterns in the newborns of smoking mothers is unknown and may provide insight into the role of vitamin C supplementation in respiratory outcomes of the newborn.

In this issue of the *Journal*, Shorey-Kendrick and colleagues (pp. 745–755) report their analysis of the relationship between vitamin C supplementation in pregnant smokers and DNA methylation changes in the offspring (17). Targeted bisulfite sequencing was performed in the placenta, cord blood, and buccal mucosa from subjects previously recruited into the above-mentioned randomized controlled trial (15) to assess for differentially methylated CpG sites and differentially methylated regions in genes previously identified as impacted by IUS exposure. These “at-risk” sites were then analyzed to determine the effect of maternal vitamin C supplementation on their methylation. Finally, CpG sites whose methylation was significantly associated with maternal smoking, yet restored by vitamin C supplementation, were then examined for association with measures of newborn pulmonary function and wheeze at 1 year of age.

The authors identified significant DNA methylation differences between the offspring of nonsmoking mothers and placebo-treated smoking mothers in all three tissues. Using hierarchical clustering, they also demonstrated that the methylation patterns of subjects born to vitamin C-supplemented smokers were more similar to nonsmoking mothers than placebo-treated smokers. Furthermore, they demonstrated that 203 CpG sites were restored back to at least 50% of the nonsmoking values among all three tissues, suggesting that vitamin C supplementation in pregnant smokers results in a reduction in smoking-related methylation changes across the genome. Interestingly, the reversal pattern

in DNA methylation levels with vitamin C supplementation persisted in the buccal epithelium 3 to 6 years after birth. Using data from a recently published meta-analysis of cord blood methylation (7), the authors were able to replicate 35 CpGs that fell within 24 unique differentially methylated regions with a consistent direction of association. Notably, the authors identified significant enrichment for restored CpGs associated with pulmonary function at birth.

This study has several strengths that make it a significant addition to the rapidly growing literature on the impact of maternal smoking on newborn DNA methylation patterns. First, by demonstrating that vitamin C supplementation in pregnant smokers counteracts a proportion of the methylation impact of IUS, the authors provide insight into potential biologic mechanisms that may explain the improved neonatal respiratory outcomes observed with vitamin C supplementation. The authors provide further evidence supporting this potential mechanism by demonstrating that vitamin C modulates the methylation of CpGs associated with infant pulmonary function and wheeze. Finally, the persistence of epigenetic changes in the buccal mucosa of children aged 3 to 6 years demonstrates the possible lasting impact of vitamin C supplementation on long-term respiratory outcomes in the offspring of smoking mothers.

Several limitations to the current study are worthy of mention. First, the sample size for each of the tissue types is quite small, restricting the power to detect differential methylation. In addition, the ability to address additional confounders that are inherent in developmental data, including cell-specific methylation changes, the impact of additional prenatal and postnatal environmental perturbations, and other factors such as sex and race, is limited. Furthermore, interrogation of an *a priori* set of genes limits the ability to detect novel associations. Although it is beyond the scope of the current study, an epigenome-wide analysis of the impact of vitamin C supplementation in a larger number of pregnant smokers would be critical to assess the true impact of vitamin C supplementation on the DNA methylation signature of the newborn.

Although the results of this study provide proof-of-concept evidence for a mechanistic link between vitamin C supplementation in pregnant smokers and improved neonatal respiratory outcomes through its impact on DNA methylation, additional investigation is needed to fully understand the biologic basis of these associations. Importantly, the results of the current study should motivate additional investigation of the impact of vitamin C supplementation of pregnant smokers on the newborn methylation signature as part of the investigators’ ongoing larger randomized clinical trial, “Vitamin C to Decrease the Effects of Smoking in Pregnancy on Infant Lung Function” (NCT01723696). ■

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Patient-centered Outcomes in Idiopathic Pulmonary Fibrosis Clinical Trials

In 2018, we will mark a decade of commercially available therapy for idiopathic pulmonary fibrosis (IPF)—pirfenidone was approved for use in Japan in 2008. Since that time, evidence of the efficacy of both pirfenidone and nintedanib in IPF has continued to accumulate. Five large phase 3 clinical trials (CAPACITY I and II, ASCEND, and INPULSIS I and II) have provided convincing evidence that these two drugs slow disease progression as defined by a reduction in the rate of FVC decline over 1 year (1–3). These findings have been appropriately lauded, and the U.S. Food and Drug Administration has also weighed in, emphasizing the now-recognized primacy of FVC as the preferred primary endpoint in IPF clinical trials, noting that “the relationship between FVC and mortality trends in both sets of clinical trials strengthened our ability to rely on FVC as a clinically relevant efficacy measure in IPF” (4).

Nevertheless, the choice of primary endpoint in IPF trials has been subject to additional nuanced debate (5, 6). Although declines in FVC over time likely do represent progression of IPF in many cases and indeed may be distressing to patients

and their healthcare providers, FVC is simply a physiological measurement and not a “patient-centered” outcome. A pharmacologically induced reduction in the rate of FVC decline has no tangible benefit from the patient’s perspective. Evidence that antifibrotic therapy positively influences one or more patient-centered outcomes would have both a meaningful impact on patient care and important implications for future clinical trials in IPF.

In this issue of the *Journal*, Ley and colleagues (pp. 756–761) provide new evidence that directly addresses this knowledge gap (7). They performed randomized comparisons of pirfenidone versus placebo using data from three phase 3 trials of pirfenidone (the two CAPACITY trials and ASCEND). They examined the effect of allocation to a pirfenidone arm on the risk of respiratory hospitalization, nonrespiratory hospitalization, and all-cause hospitalization using time-to-event analyses. They found that pirfenidone was associated with a lower risk of respiratory-related hospitalization at 1 year (a 5% absolute risk reduction, which corresponds to a number needed to treat of 20) but was not associated with all-cause or nonrespiratory hospitalizations. Pirfenidone was also associated with a lower

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