Stress: A Possible Link between Genetics, Epigenetics, and Childhood Asthma

According to the World Health Organization, approximately 235 million people suffer from asthma. Asthma is the most common chronic disease in childhood and is influenced by societal factors (1). Death related to asthma is more likely in countries with limited resources. Puerto Rican children are particularly burdened, with over three times the risk of asthma of non-Hispanic whites. It is likely that both genetic and environmental factors contribute, but known genetic variation accounts for only a small proportion of asthma risk (2). The dominant host and environmental factors that increase asthma risk in Puerto Rican children remain poorly understood.

Psychosocial stress has emerged as an important factor in the pathogenesis of childhood asthma (3). Puerto Rico has a high rate of homicide, resulting in increased exposure of Puerto Rican children to violence, contributing to a high prevalence of post-traumatic stress disorder (PTSD). Both PTSD and psychosocial stress have been associated with increased asthma morbidity, but mechanisms that link psychosocial stress and childhood asthma remain elusive.

The genetic contribution to risk for developing PTSD is estimated to be approximately 30% (4). *ADCYAP1R1* encodes a membrane receptor for pituitary adenylate cyclase–activating peptide, and both are highly expressed in the hypothalamus and limbic structures that are integral to the stress response. Genetic variation of *ADCYAP1R1* increases the risk of PTSD in women (5), including those who experienced past childhood maltreatment (6). The polymorphic variant associated with PTSD is located within an estrogen-responsive element within *ADCYAP1R1*, offering a potential explanation for the effects observed in females. Although this association has not replicated in all cohorts (7), genetic variation within *ADCYAP1R1* provides a potential link between host genetics and PTSD. In this volume of the *Journal*, Chen and colleagues (pp. 584–588) report an association between the same *ADCYAP1R1* single-nucleotide polymorphism as identified in PTSD and childhood asthma in Puerto Ricans, providing new insight into the stress–asthma connection (8). This finding supports that *ADCYAP1R1* is associated with airway disease, but does not address how psychosocial stress modifies disease risk.

Epigenetic modifications, including methylation of CG cytosines, are another potential contributory component to asthma risk. Methylation patterns throughout the genome are essential for guiding appropriate spatial and temporal expression of the transcriptome. These patterns are established during gametogenesis and early postfertilization development through a process of sequential reprogramming that includes erasure and reestablishment (9). Once originated, patterns of DNA methylation are transmitted through somatic cell division and are thought to be stable. However, discoveries in cancer have shown that losses and gains in site-specific DNA methylation occur despite the presumed stability. Furthermore, these changes are highly relevant to disease etiology (10). Other research has concurrently focused on populations of individuals who experienced adverse early life conditions, and linked this to increased risk for developing chronic disease as adults (11). This is now referred to as the "early origins of adult disease" hypothesis, and epigenetic mechanisms have been invoked as a potential explanation. Studies in mice support that early-life exposures can modify epigenetic marks and alter severity of allergic airway disease (12). Chen and colleagues considered whether alteration in epigenetic programming of *ADCYAP1R1* was associated with risk of childhood asthma.

Associations between altered methylation at specific candidate genes and risk of asthma have previously been reported, and stress is known to induce changes in methylation in both animal models and humans. Chen and colleagues have for the first time demonstrated that these relationships are intertwined. They found that increased methylation of a single CpG in the *ADCYAP1R1* promoter is associated with increased risk of asthma, particularly in children who were exposed to violence (8). The implications of these findings suggest that exposure to trauma in childhood specifically changed *ADCYAP1R1* methylation that, in turn, alters transcriptional regulation of this gene in a manner that increases asthma risk. These observations also suggest that the early life environment could both have a lasting impact on development of complex disease and reprogram subsequent biological response to physiological stress.

Plausibility for exposure to stress impacting DNA methylation and long-term phenotypic consequences comes from previous studies in rats. Rat pups exposed to low levels of maternal nurturing show higher cytosine methylation of a single CpG site in the glucocorticoid receptor promoter as compared with low methylation of the same CpG in pups receiving high levels of maternal care. This change occurs in the hippocampus (13) and shapes lifelong patterns of stress response in the pups.

Although the observations by Chen and colleagues are novel, results should be interpreted with some caution. DNA methylation levels were measured in white blood cells, leaving unanswered the relevance of this finding to the affected tissue. It also remains unclear whether altered methylation is cause or consequence. One possibility is that altered methylation results from skewing of epigenetic reprogramming during embryonic development, prior to tissue differentiation. If so, it is conceivable that the shifted pattern would be evident in many tissues due to somatic heritability of DNA methylation patterns. The methylation change could thus be considered as contributing to potentiation of the stress response and PTSD, increasing asthma risk. Although not supported by current evidence, another possibility is that a methylation profile shift occurred in response to stress and that this change both is present in a sufficient number of blood cells to enable detection and simultaneously occurred in the relevant affected tissues. Prospective studies would help resolve these possibilities. Also unclear is whether or not the reported changes in genetic variation or CpG methylation are functionally relevant, resulting in altered gene expression. Replication studies in additional cohorts are needed to bolster these results.

Despite the limitations, findings reported by Chen and colleagues provide novel insight into the complex relationship between the external environment and host factors that regulate pathogenesis of childhood asthma. The results suggest that both epigenetic and genetic alterations in *ADCYAP1R1* link psychosocial stress to childhood asthma. Studies focused on the complex relationship between common environmental exposures, epigenetic programming,

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and host genetics will likely provide additional insight into the pathogenesis of childhood asthma.

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T-Cell Immunotherapy in Cystic Fibrosis
Weighing the Risk/Reward

Cystic fibrosis (CF) is an autosomal recessive disorder resulting in abnormalities of the protein known as the cystic fibrosis transmembrane conductance regulator (CFTR). There are numerous gene mutations that cause cystic fibrosis, the most common defect being deltaF508, named for its deletion of three nucleotides resulting in the absence of phenylalanine at codon 508 (1). Individuals with CF have alterations in airway surface liquid and impairment of mucociliary clearance leading to chronic airway infection and inflammation. Pulmonary infection and inflammation lead to a progressive decline in lung function, and death is most often a result of respiratory failure (2). A complex relationship exists between infection and immunity in the CF lung, with patients experiencing an exaggerated immune response. This response includes excessive airway neutrophilia and submucosal lymphocytosis. There is an emerging role for adaptive immunity in CF with the discovery that sputum of patients with CF has increased levels of T-helper 17 (Th17) cell–associated cytokines, IL-17 and IL-23. In addition, IL-17–producing cells are present in the airway submucosa (3).

Common infectious pathogens in patients with CF include *Pseudomonas aeruginosa* and *Aspergillus fumigatus*. *P. aeruginosa* infection occurs at an early age in CF and results in a more rapid decline in lung function and decreased survival (4–6). There are multiple methods by which *P. aeruginosa* evades the immune system, contributing to its virulence. *A. fumigatus* is a fungal pathogen isolated from sputum of patients with CF, and observational studies suggest increased prevalence in patients using chronic inhaled antibiotics (7, 8). Patients can either be colonized with *A. fumigatus* or develop an immune-mediated hypersensitivity to *Aspergillus*, known as allergic bronchopulmonary aspergillosis (ABPA). The impact of *A. fumigatus* colonization remains unclear, although a recent pilot randomized control study found that itraconazole treatment did not provide any clinical benefits (9). ABPA is associated with a decline in lung function (10). There may be potential benefit from early intervention against these pathogens, either through the use of antimicrobials or immunomodulators.

In this issue of the *Journal*, two articles address the role of IL-17 and Th17 cells in the CF lung. Tiringer and colleagues (pp. 621–629) examined T-cell cytokine profiles in bronchoalveolar lavage fluid and lung explants from patients with CF and control subjects (11). This study shows that IL-17 (a Th17 cytokine) and IL-5 and IL-13 (Th2 cytokines) are increased in patients with CF undergoing exacerbation versus stable CF. These cytokines positively correlated with high-resolution computed tomography radiographic changes. Further, IL-17 levels negatively correlated with FEV_1, suggesting that elevated Th17 presence in CF is related to declining lung function. Data from this study are in agreement with early work that showed elevated IL-17 in early CF (median age 1.7 years), which was further increased in established CF (median age 9.3 years) (12). Tiringer and colleagues also report increased IL-17$^+$ staining T cells similar to the recent observation of elevated CD4$^+$ Th17 cells and γδT cells in both the lung and lymph nodes of patients with CF (12, 13). Interestingly, elevated IL-17 was predictive of future acquisition of *P. aeruginosa* infection. IL-17 has been previously shown to be elevated in patients with CF who have ongoing *P. aeruginosa* infection, and IL-17 was reduced by antibiotic treatment (14). The reasons for elevated IL-17 prior to *P. aeruginosa* colonization are unclear. Classically, the onset of *P. aeruginosa* infection in CF has been temporally linked to viral infection (15, 16). Further, *Staphylococcus aureus* infection, a known inducer of IL-17, often precedes *P. aeruginosa* in CF (17, 18). It is possible that IL-17 elicited in response to other pathogens in the CF lung