[Original Research]

CHEST

An Integrative Transcriptomic and Metabolomic Study of Lung Function in Children With Asthma

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BACKGROUND: Single omic analyses have provided some insight into the basis of lung function in children with asthma, but the underlying biologic pathways are still poorly understood.

METHODS: Weighted gene coexpression network analysis (WGCNA) was used to identify modules of coregulated gene transcripts and metabolites in blood among 325 children with asthma from the Genetic Epidemiology of Asthma in Costa Rica study. The biology of modules associated with lung function as measured by FEV_1, the FEV_1/FVC ratio, bronchodilator response, and airway responsiveness to methacholine was explored. Significantly correlated gene-metabolite module pairs were then identified, and their constituent features were analyzed for biologic pathway enrichments.

RESULTS: WGCNA clustered 25,060 gene probes and 8,185 metabolite features into eight gene modules and eight metabolite modules, where four and six, respectively, were associated with lung function ($P \leq .05$). The gene modules were enriched for immune, mitotic, and metabolic processes and asthma-associated microRNA targets. The metabolite modules were enriched for lipid and amino acid metabolism. Integration of correlated gene-metabolite modules expanded the single omic findings, linking the FEV_1/FVC ratio with *ORMDL3* and dysregulated lipid metabolism. This finding was replicated in an independent population.

CONCLUSIONS: The results of this hypothesis-generating study suggest a mechanistic basis for multiple asthma genes, including *ORMDL3*, and a role for lipid metabolism. They demonstrate that integrating multiple omic technologies may provide a more informative picture of asthmatic lung function biology than single omic analyses. CHEST 2018; ■(■):■-■

Q9 **KEY WORDS:** asthma; integrative omics; lung function; metabolome; transcriptome

ABBREVIATIONS: BDR = bronchodilator response; CAMP = Childhood Asthma Management Program; HILIC = hydrophilic interaction liquid chromatography; QC = quality control; SNP = single-nucleotide polymorphism; WGCNA = weighted gene coexpression network analysis Q3

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Asthma, a disorder characterized by variable and reversible airway obstruction, hyperresponsiveness, and inflammation, represents one of the most common chronic conditions among children and adults worldwide.[1,2] Asthmatic lung function abnormalities are present early in life,[3,4] track through childhood and adulthood,[5,6] and are strong determinants of disease exacerbations and severity.[7,8]

Reduced lung function in patients with asthma is thought to emerge from complex gene-environment interactions.[9] Advances in high-throughput technologies allow us to explore such interactions at the level of the epigenome, genome, transcriptome, proteome, and metabolome. Combining the transcriptome, which reflects genomic activity, with the metabolome, which is sensitive to environmental influences and closely related to phenotype, may be particularly informative. Although previous studies have investigated metabolomic and transcriptomic profiles of asthma separately, to date only two studies, with limited sample sizes, have integrated the two omes together in humans.[10,11] Relative to the use of single omics technologies, this integrative approach demonstrated increased predictive ability for asthma and its subtypes, and greater biologic insights. Consequently, integrative omics represents an exciting new avenue in asthma research.[12]

Currently, there are no analytic standards for integrative omics. However, network medicine, a rapidly emerging field that moves away from reductionist methodologies to combine systems biology and network science, represents a particularly promising approach. It provides a holistic methodology to better understand disease through the identification and investigation of nonlinear relationships and networks of interacting components. This provides insights into these conditions beyond the level of a single gene or omic platform. Weighted gene coexpression network analysis (WGCNA) is a network Q10 method for identifying clusters or modules or highly correlated variables (eg, genes, metabolites) that are likely to be coregulated, or working together in a biologically coherent fashion. A module can then be summarized as a single unit, which can be correlated with phenotypes or other modules of interest.

The aim of this study was to conduct an integrated analysis of the blood transcriptome and metabolome among children with asthma participating in the Genetic Epidemiology of Asthma in Costa Rica[13] cohort to identify biologically informative networks of genes and metabolites associated with asthmatic lung function. The Genetic Epidemiology of Asthma in Costa Rica cohort recruited children with mild-to-moderate asthma from the Central Valley of Costa Rica. This area represents a Hispanic population isolate which is genetically homogenous and has one of the highest prevalences of asthma in the world (24% in children),[14] making it uniquely suited for the exploration of the integrative omic underpinnings of asthmatic lung function. In particular, the study focuses on FEV_1 and FEV_1/FVC ratios, which are thought to mediate the association between early life characteristics and asthma.[15]

Methods

Study Population

This integrative omic study was nested within the Genetic Epidemiology of Asthma in Costa Rica Study,[13] which recruited children 6 to 14 years of age with mild-to-moderate asthma and their parents from the Central Valley of Costa Rica. Children were eligible if they had physician-diagnosed asthma and at least two episodes of respiratory symptoms or asthma attacks in the prior year, and a high probability of having six or more great-grandparents born in the Central Valley of Costa Rica.[16,17] A total of 1,165 children with asthma were enrolled in the original study. All children completed a protocol at enrollment, including questionnaires, spirometry, and collection of blood when children were not exacerbated. Most blood samples were processed within 4 h; RNA was extracted and stored in PAXgene tubes. Genome-wide single-nucleotide polymorphism (SNP) Q11 genotyping and RNA expression profiles were generated for a subset of the children with suitable samples. Genotype data were obtained with TaqMan real-time polymerase chain reaction with an ABI Prism 7900 machine (Applied Biosystems).[18] Standard manufacturer-recommended polymerase chain reaction conditions were used. Children were prioritized for metabolomic profiling if they had both genome-wide genetic and genome-wide expression data, with the goal of conducting integrative omic analyses. Children with both metabolomic and transcriptomic profiling were included in the current study. Written parental and participating child consent was obtained. The study was approved by the Partners Human Research Committee at Brigham and Women's Hospital (Boston, MA; protocol No. 2000-P-001130/55) and the Hospital Nacional de Niños (San José, Costa Rica).

Lung Function

At enrollment, baseline lung function was investigated by spirometry (FEV_1 and FEV_1/FVC ratio), bronchodilator response (BDR) (percentage difference in FEV_1 from baseline after inhaled albuterol), and airway responsiveness to methacholine (determined as the provocative dose of methacholine resulting in a 20% drop in FEV_1 from baseline) (e-Appendix 1). Q12

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