More Evidence for Inborn Dysregulation of Sphingolipid Metabolism in Children with Asthma?

Since the initial identification of 17q21 as an asthma susceptibility region for childhood asthma (1), much attention has focused on how factors regulated and expressed in this region relate to childhood asthma's pathogenesis. ORMDL3 has probably attracted the most attention, with increased expression predicted to be associated with many of the 17q21 asthma-risk alleles. Early ORMDL3 overexpression and knockout studies suggested that inflammation, mainly through activation of the unfolded protein response, could be the functional link to asthma (2). The identification of ORMDL3 as a regulator of sphingolipid *de novo* synthesis 3 years after the initial genome-wide association study suggested that this lipid class of mainly membrane constituents and some signaling molecules may also be involved in asthma pathogenesis (3). Elucidating a role for genetically altered sphingolipid metabolism in asthma has been challenging, especially as the increased expression of ORMDL3 predicted an inhibition of sphingolipid synthesis. However, progress has slowly been made.

Longitudinal birth cohorts with metabolomic profiling are well suited to address questions regarding the early metabolic environment, potentially revealing important clues into alterations of biochemical pathways, such as sphingolipid metabolism, associated with disease development. However, metabolomic analyses present challenges by the sheer number of comparisons made, the inherent collinearity among individual metabolites, and by the nature of obtaining a momentary "snapshot," susceptible to environmental influences over time. Finding additional means of validation and replication is imperative for the interpretation of results and understanding whether identified associations can be considered valid and appropriate.

In this issue of the *Journal*, Rago and colleagues (pp. 853–863) use a combination of a well-characterized, prospective longitudinal replication cohort and statistical modeling to address these challenges (4). The authors analyzed sphingolipids by untargeted plasma metabolomics from children in the $COPSAC_{2010}$ (Copenhagen Prospective Studies on Asthma in Childhood 2010) cohort at age 6 months and 6 years, and they investigated the association of sphingolipid profiles with the development of asthma symptoms by age 3 and measures of lung function at age 6. Also, mRNA expression of subunits of serine-palmitoyl-transferase (SPT), the rate-limiting enzyme for sphingolipid *de novo* synthesis, and ORMDL3 from nasal brushings and genotyping at 17q21 SNPs were performed. To test whether the $COPSAC_{2010}$ cohort findings could be replicated, the investigators used plasma samples from the VDAART (Vitamin D Antenatal Asthma Reduction Trial) study, assessing the relationship between metabolic profiles from children at age 1 and the emergence of respiratory symptoms by age 3. Reduced levels of four sphingolipids, ceramide glycosyl-*N*-stearoyl-sphingosine (d18:1/18:0), stearoyl sphingomyelin (d18:1/18:0), sphingomyelin (d18:1/20:1, d18:2/20:0), and sphingomyelin (d18:1/18:1, d18:2/18:0), were found to be associated with an increased risk of asthma at age 3. Although these findings did not reach significance beyond the false discovery rate threshold, using a replication cohort to confirm the association of these sphingolipids at 6 months and 1 year with early-onset asthma provides some evidence that this pathway is associated with the early emergence of asthma symptoms. The inverse association of these sphingolipid levels to asthma risk is in accordance with the assumption that lower sphingolipid synthesis is associated with asthma. However, the metabolome at age 6 months was not associated with asthma at age 6 years, with no correlations between the metabolomes from 6 months and 6 years. Nevertheless, these findings support data in older children with asthma who had lower sphingolipid blood levels than children without asthma (5). These findings also fit with recent metabolic profiling of maternal plasma during the third trimester of pregnancy, showing lower levels of sphinganine-1-phosphate and *N*-palmitoyl-sphingadienine negatively associated with asthma risk in the offspring (6).

A novel contribution of this study is the association of sphingolipids to lung function data. Two phosphosphingolipids, sphinganine-1-phosphate and sphingosine-1-phosphate, were negatively associated with increased airway resistance at 6 years. Although not significant beyond the false discovery rate threshold, consistent findings were replicated using a partial least squares discriminant analysis model. However, sphingolipids were not associated with other lung function parameters, such as FEV_1 or methylcholine challenge. Interestingly, the authors found an interaction of asthma 17q21 asthma-risk allele of rs12936231 with low sphinganine-1-phosphate and high airway resistance. Despite the noted limitations, these findings connect low sphingolipid levels to airway reactivity in children for the first time. This additionally confirms experimental studies using airway models, demonstrating that decreased sphingolipid synthesis through reduced SPT expression or activity results in increased airway reactivity (7). Although experimental SPT inhibition models may be too reductionist, ORMDL3 overexpression or knockout cell and mouse models have additionally had challenges defining functional consequences of increased ORMDL3 in asthma (8). This is likely attributed to the complexity of *de novo* sphingolipid synthesis regulation, a fine-tuned system that depends on the exact stoichiometry of ORMDL3 and SPT (9).

Another interesting and novel aspect of the study is the association of the nasal cell gene expression of SPT and ORMDL3 to 17q21 asthma-risk genotypes. The authors show associations of large and small SPT subunits, including Sptlc1 and Sptssa, with 17q21 genotypes, although interactions with SPT and the two phosphosphingolipids were less revealing. Interestingly, the authors

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found no association between nasal ORMDL3 gene expression and 17q21 genotype. Nevertheless, these findings should inform further studies on the regulation and role of SPT subunits in asthma.

Overall, this study lends evidence supporting the concept that genetically altered sphingolipid metabolism in children who carry 17q21 asthma-risk genotypes may lead to functional consequences on airway resistance, acting as a predisposing factor for the development of asthma. Although the manifestations of classic inborn disorders of sphingolipid metabolism mainly result from the accumulation of toxic products affecting the nervous system and skin, decreased synthesis of bioactive lipids such as sphinganine-1-phosphate and sphingosine-1-phosphate may also have specific functional consequences (10). We may also learn from other manifestations of decreased SPT activity, such as in hereditary sensory autonomic neuropathy caused by a loss of function mutation of the SPT subunit Sptlc2, which has recently been shown to have relevant consequences on immune cell function (11). Although the exact role of sphingolipids in asthma remains enigmatic, Rago and colleagues have opened the door a little further, providing another glimpse of how this class of lipids is involved in the complex pathogenesis of childhood asthma. ■

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Understanding How Asthma Starts: Longitudinal Patterns of Wheeze and the Chromosome 17q Locus

Most childhood asthma starts in the preschool years. Symptoms such as wheeze, cough, and dyspnea are not specific to asthma and can also represent transient symptoms due to viral respiratory tract infections. Triggers of preschool wheeze can change over time (1) and therefore are not reliable predictors of asthma. No valid, reproducible diagnostic or predictive test of preschool asthma is currently available (2). This inability to diagnose preschool asthma has seriously impeded better understanding of childhood-onset asthma and the ability to design targeted, early-life interventions.

In this issue of the *Journal,* Hallmark and colleagues (pp. 864–870) combine two strategies to better understand the development of childhood wheeze: the description of longitudinal patterns of wheeze in seven U.S. birth cohorts participating in the Children's Respiratory Research and Environment Workgroup, and the investigation of the association of these longitudinal wheezing phenotypes with the 17q12-21 locus ("17q") (3). 17q is the most replicated childhood-onset asthma locus (4). Using data from birth until age 11 years, their latent class modeling revealed four classes of wheeze: infrequent (no or low presence of wheeze;

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