A Long Noncoding RNA "lnc"ed to Asthma Genetics

Genome-wide association studies (GWAS) over the past 15 years have overwhelmingly established asthma as a genetic disease by identifying numerous variants, including SNPs, in multiple genomic loci that are associated with asthma. These association studies, however, do not establish causal relationships between the variants and asthma and thus cannot tell us *how* the genetic variants contribute to asthma pathogenesis. A major effort in post-GWAS asthma research thus is focused on identifying potentially causal variants in genes and understanding their roles in asthma pathogenesis through functional, mechanistic studies. Such effort is beginning to yield results that provide new mechanistic insights. For example, studies on the variants in the 17q21 locus—the most significant and highly replicated asthma GWAS signal—have highlighted functional roles of the *ORMDL3* and *gasdermin B* (*GSDMB*) genes as well as their variants in phenotypes such as airway inflammation and epithelial pyroptotic cell death that are relevant to asthma pathogenesis (1–4). These studies focus mostly on protein-coding genes but not on noncoding sequences or loci, where the majority of asthma-associated variants are located. Thus, noncoding loci that harbor many of the asthma variants are still awaiting functional examination. In this issue of the *Journal*, Li and colleagues (pp. 283–292) report on their investigation of the asthma-associated variants in the 5q31.1 locus (5). Unexpectedly they identified a long noncoding RNA (lncRNA), *TH2LCRR* (T helper type 2 locus control region-associated RNA), as a potential causal link between the 5q31.1 variants and asthma susceptibility (5).

Li and colleagues focused on the asthma SNPs in the chromosome 5q31.1 region, which, like the 17q21 locus, has long been identified as an asthma risk locus (6). One of best studied variants in 5q31.1 (rs20541) is a nonsynonymous SNP (R110Q) that results in higher activity of IL-13, a central mediator of allergic inflammation (7). Although *IL-13* is upregulated in patients with asthma and has been shown to mediate asthma-like symptoms in animal models, results of clinical studies targeting IL-13 and its receptor have so far been inconsistent (8). In addition to rs20541, there are several other 5q31.1 SNPs that are associated with asthma, albeit with uncertain functional basis. Rs1295686, a noncoding SNP that is in linkage disequilibrium with rs20541, is significantly associated with asthma in a Caucasian population and in children (9, 10). Moreover, several less-studied SNPs are in strong linkage disequilibrium with rs1295686 and rs20541. Li and colleagues examined whether these additional SNPs in the 5q31.1 region represent functional variants that could influence asthma susceptibility. Using a reporter-based luciferase assay, they found that a haplotype block containing three SNPs (rs1295685, rs848, and rs847) in the 5q31.1 locus drives the upregulation of a recently identified lncRNA (*TH2LCRR*). Interestingly, *TH2LCRR* was previously shown to regulate the expression of proinflammatory Th2 cytokines, including IL-13 (11, 12).

Using functional genomics and molecular biology approaches, the authors provide further evidence that rs1295685, rs848, and rs847 represent functional SNPs in 5q31.1. As these SNPs reside in the 3′-untranslated region of *IL-13*, the function of these variants was originally hypothesized to influence *IL-13* expression. However, through chromatin conformation capture assay, the authors show that the segment harboring the three SNPs instead acts as a transcriptional enhancer that interacts with the promoter of *TH2LCRR*. Using chromatin immunoprecipitation technique, the authors also demonstrate that the three SNPs increase the binding of transcription factors (TCF3, USF1, and HNF4a) to the promoter region, resulting in increased expression of *TH2LCRR*. Moreover, by mining existing RNA-sequencing transcriptomic data, the authors further show that *TH2LCRR* is upregulated in patients with asthma and in lung cells exposed to known asthma triggers, such as house dust mite antigen and rhinovirus.

By carefully examining the variants in 5q31.1 locus, Li and colleagues uncovered a previously unknown role for the lncRNA *TH2LCRR* in asthma susceptibility. Their functional studies provide evidence pointing to the potential role of this lncRNA and the associated SNPs in asthma pathogenesis. This work expands our knowledge of the functional roles of asthma-associated SNPs and genes toward a more complete understanding of this complex disease. Additionally, given that the contribution of lncRNAs in asthma is increasingly being recognized (13, 14), their findings provide further evidence supporting the emerging role of lncRNAs in asthma pathogenesis. Furthermore, as we continue to illuminate the basic functions of lncRNAs which used to be considered the "dark matter" of the genome, Li and colleagues' finding linking *TH2LCRR* to asthma susceptibility adds to our broader understanding of the diverse biological roles of lncRNAs (15).

The findings in this study are somewhat unexpected, as it was originally thought that the functional SNPs in 5q31.1 directly act on the *IL-13* gene, which resides in this chromosomal region and encodes a known mediator of inflammation. It remains unclear whether the haplotype block also affects *IL-13* expression or function, and if so, whether such effect is mediated by *TH2LCRR*, which is known to regulate *IL-13* expression (11, 12). There are additional unresolved issues. For example, the expression of *TH2LCRR* was not examined in the relevant immune cells that produce many of the cytokines, including IL-13, that are involved in the Th2 response in asthma (8). There was no experiment examining the effect of knocking down or deleting *TH2LCRR* on the inflammatory phenotype in relevant immune or airway cells. Finally, and perhaps more importantly, it remains unclear how exactly *TH2LCRR* as a

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lncRNA may exert functional effects on airway inflammation or other phenotypes relevant to asthma pathogenesis. Future studies addressing these important questions will further strengthen the functional link between the lncRNA *TH2LCRR* and asthma pathogenesis. ■

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Ronald A. Panganiban, Ph.D.
Quan Lu, Ph.D.
Department of Environmental Health
Harvard T.H. Chan School of Public Health
Boston, Massachusetts

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