

Assessing Asthma Medication Responses in U.S. Minority Children by Whole-Genome Sequencing

Asthma associated with environmental exposures and multiple genetic factors is prominently linked to immunological mechanisms (1). Life-threatening symptoms of asthma are related to airflow limitation in the airways, and inhaled β_2 -agonists constitute the preferred first-line bronchodilator treatment used to rapidly restore the patient's acute airflow restrictions. Bronchodilators' immediate effects in lung function vary widely between individuals and populations. The U.S. populations with the lowest bronchodilator response (BDR) also associate with the highest asthma prevalence and mortality (2).

In the last 10 years, genome-wide association studies (GWASs) have paved the way in the hunt for common risk factors underlying complex diseases, which have contributed to improving biological understanding of disease and revealing novel therapeutic targets (3). However, despite the large sample size analyzed and the number of studies involved, less than 5% of variance in asthma liability is explained by known loci to date (1), indicating a paucity of asthma risk loci detected by GWASs. The knowledge is even narrower if the focus is on the genetics of asthma treatment responses, where a few GWASs have been completed so far. Considerations of the observation that common variants generally explain a minor proportion of complex trait heritability (4) are abundant, and the proposed explanations are diverse. These include the potential role of rare variants with large effects that are not targeted by GWASs to date (5).

In this issue of the *Journal*, Mak and colleagues (pp. 1552–1564) (6) aimed to clarify the respective influence of common and rare variants on BDR in U.S. minority children by performing the first GWAS based on whole-genome sequencing (WGS) data. The study also involved transcriptomics in subsets of samples and chromatin immunoprecipitation sequencing in primary bronchial smooth muscle cells. To maximize study power, Mak and colleagues (6) selected approximately equal numbers of patients from three minority populations (Puerto Ricans, Mexicans, and African Americans) from both tails of the BDR distribution, summing up a total of 1,441 children with asthma who were subjected to WGS. Population-specific analyses of common variants were unsuccessful in identifying significant associations. However, a transethnic analysis of common variants unearthed associations for 10 novel loci explaining between 16% and 23%

of variation in BDR when considered together in a model. Unfortunately, the model was not tested in independent patients, and as a consequence, the estimates should be taken with caution. The top hit is located near *DNAH5-LINC01194* (encoding dynein axonemal heavy chain 5 and long intergenic non-protein-coding RNA 1194, respectively), a locus previously linked to chronic obstructive pulmonary disease and immunoglobulin E levels (7, 8). Interestingly, one novel and likely functional variant is located in the *NFKB1* gene encoding the subunit 1 of NF- κ B (nuclear factor κ B), a central transcription regulator of immune functions. The *NFKB1* allele associated with low BDR was found more prevalent among African populations, which may serve to partially explain the relationship between African ancestry and the lower BDR in the U.S. populations (2). Strikingly, despite the attractive evidence and the fact that several other genes flanking the association hits were previously known to have lung-phenotype associations, the authors were unable to replicate the findings in five independent studies. This was not unexpected, according to the authors, given the lack of age- and population-matched asthma replication cohorts of sufficient sample size, as well as the well-known genetic differences between populations (9) and between childhood and adult asthma (10). Association tests combining common and rare variants yielded five additional regions that were associated with BDR in specific populations or in combined analyses, explaining 4–8% of the variation in BDR. However, most genes near these five loci had no known functional relationships with BDR. Overall, it is surprising to find that none of the loci unearthed by the study had a direct connection with β_2 -adrenergic receptor signaling pathway, even though they did with lung function and the allergic response.

The strengths of the study are several and necessary to provide findings that were consistent with the biology of the trait, including the multi-omics approach, the power maximization providing the huge resources necessitated to complete robust WGS at the required scale, and the focus on diverse minority populations with extensive clinical characterization. On the downside, the study exemplifies the obstacles of applying WGS in complex traits at the present time: most prominently, the sacrifice of study power, the scarcity of independent studies with sequencing data available for replication, the lack of gold standard bioinformatics and statistical methods to deal with all types of genetic variants and variant grouping strategies, and the limited understanding of sequence element functions and their genome reach to help interpreting the results.

There is currently a debate around how much of the genetic heritability of complex traits is caused by rare variants. Although replication studies are still necessary, Mak and colleagues (6) provide novel loci in which both common and rare variants could be contributing a large proportion of the genetic variation in BDR. ■

J.M.L.-S. is supported by Cabildo Insular de Tenerife and Consejería de Educación y Universidades. C.F. is supported by Instituto de Salud Carlos III, Madrid, Spain (PI14/00844 and PI17/00610) and cofinanced by the European Regional Development Funds, "A way of making Europe," from the European Union. The funding agencies had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Originally Published in Press as DOI: 10.1164/rccm.201803-0457ED on March 26, 2018

Author disclosures are available with the text of this article at www.atsjournals.org.

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Early Returns in Vascular Inflammation in ARDS

Activation of intravascular inflammatory cells is believed to be a central facet of the complex pathophysiology of acute respiratory distress syndrome (ARDS) and the acute alveolar–capillary injury that is characteristic of this syndrome, as is triggering of pivotal biochemical pathways (1). In an article in this issue of the *Journal* (2), Abdunour and an interinstitutional group of coinvestigators (pp. 1575–1585) report determinations of leukocyte numbers and lipid mediator concentrations in peripheral blood from patients enrolled in a prospective, multicenter study of aspirin (ASA) for prevention of ARDS, the LIPS-A (Lung Injury Prevention Study with Aspirin) trial (3). The protocol required blood to be collected from subjects at risk at baseline, after randomization, and before administration of ASA or placebo or development of ARDS, and serially over 3 days, generating samples for the current analysis. The results of biologic determinations in blood collected before development of ARDS is one of the most important features

of this report (2). In studies in the field, samples are usually collected after patients meet consensus diagnostic criteria and the syndrome is established. Many of the concepts regarding evolution of ARDS from a pathologic trigger to inflammatory acute respiratory failure are based on measurement of markers in the exudative phase (1) rather than in the prodrome. Early biologic information in this field and in clinical investigation of other disorders—like reliable early returns in elections—is often difficult to come by. In this case, early collection of relevant samples was based on application of a lung injury prediction score in screening patients at risk in the emergency room (2). An unexpectedly low number of subjects developed ARDS, however, limiting conclusions from the original trial (3) and this follow-up study.

Baseline plasma concentrations of 15-epi-LXA₄ (aspirin-triggered lipoxin A₄; ATL), total and intermediate monocyte numbers, and monocyte–platelet aggregates (Mo-PAs) were associated with the development of ARDS (2). The increased levels of ATL at baseline in subjects at risk are intriguing. Generation of ATL is induced by ASA (2), but patients already taking

Originally Published in Press as DOI: 10.1164/rccm.201804-0670ED on May 21, 2018