routinely investigate for these (e.g., BAL) in the National Health Service unless there are suggestive symptoms. Moreover, MLNs associated with infection or autoimmune disease would often be associated with parenchymal changes on CT. We anticipate that future studies will include longitudinal MLN measurements, perhaps with artificial intelligence, to identify any potential relationships with clinical features especially with biologics.

In conclusion, larger MLNs are associated with significantly worse spirometry and airway oscillometry values together with a higher burden of total IgE concentration in patients with persistent asthma. ■

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Correspondence and requests for reprints should be addressed to Rory Chan, M.B. Ch.B., Ph.D., Scottish Centre for Respiratory Research, Ninewells Hospital and Medical School, University of Dundee, Dundee DD1 9SY, United Kingdom. Email: rchan@dundee.ac.uk.

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Genome and Transcriptome-Wide Association Study of Fibrotic Sarcoidosis in European Americans

Shu-Yi Liao[1,3,4], Lisa A. Maier[1,3,4], and Tasha E. Fingerlin[1,2,3,4]

[1]Department of Medicine and [2]Department of Immunology and Genomic Medicine, National Jewish Health, Denver, Colorado; and [3]Department of Medicine and [4]Colorado School of Public Health, University of Colorado Denver – Anschutz Medical Campus, Aurora, Colorado

ORCID ID: 0000-0001-9477-3837 (S.-Y.L.).

To the Editor:

Although sarcoidosis may be a relatively benign disease in some, it can result in fibrosis and a generally poor and potentially fatal outcome. Unlike idiopathic pulmonary fibrosis (IPF), which predominantly affects European Americans (EAs) (1), sarcoidosis affects both EAs and African Americans (AAs), with AA fibrotic patients with sarcoidosis tending to be younger (2) and having an overall worse prognosis (3). The differential outcomes in fibrotic sarcoidosis by race may imply differential pathogenesis and/or genetic predisposition by ancestry, as noted in the article by Garman and colleagues (4), who found variants in *PVT1* associated with pulmonary fibrosis in AA sarcoidosis. Theirs is an important study highlighting the need for more genetic studies in AAs to understand the potential shared and ancestry-specific genetic risk factors for sarcoidosis and phenotypes such as fibrotic sarcoidosis. The study findings highlight the need to study larger, diverse sarcoidosis populations with specific phenotypes and incorporate other approaches to integrating data from existing studies across diverse populations, some of which are not possible for less-studied populations. These integrative approaches include discovery in a racial/ethnic group with investigation of risk genes/variants in other racial/ethnic groups, investigation of variants identified in similar diseases, and use of integrative omics approaches to define potentially functional variants. As an example, in our large EA genome-wide association study (GWAS), we identified individual variants and HLA alleles associated with sarcoidosis and, in collaboration with colleagues involved in the Garman study, tested whether those variants were associated with sarcoidosis in a large AA GWAS dataset (5). Highlighting these integrative approaches, here we *1*) investigate the frequency of a fibrotic sarcoidosis AA risk haplotype reported

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Table 1. Summary of Candidate SNPs in European American Fibrotic versus Nonfibrotic Sarcoidosis with $P_{GWAS} < 5 \times 10^{-5}$ and P_{TWAS} for Nearest Gene <0.05 in Either Whole Blood or Lung

SNP	Chr	Position	Minor Allele	Nearest Gene (±25K)	MAF	OR (95% CI)	P_{GWAS}	Blood P_{TWAS} for Nearest Gene	Lung P_{TWAS} for Nearest Gene
rs10538262	18	3188664	T	*MYOM1*	0.30	2.09 (1.52, 2.88)	5.33×10^{-6}	0.03	0.02
rs9509258	13	21081538	G	*CRYL1*	0.36	1.84 (1.39, 2.43)	1.74×10^{-5}	0.02	0.18
rs58103218	16	14521778	T	*PARN*	0.04	3.51 (1.97, 6.26)	2.16×10^{-5}	4.09×10^{-5}	0.49
rs35957277	16	69783881	C	*NOB1*	0.25	1.87 (1.40, 2.50)	2.32×10^{-5}	No data	1.90×10^{-3}
rs111660324	17	79116079	TT	*AATK*	0.16	2.08 (1.48, 2.93)	2.86×10^{-5}	0.24	0.02
rs1161662	11	129779687	C	*PRDM10*	0.11	2.29 (1.55, 3.39)	3.43×10^{-5}	2.79×10^{-3}	2.79×10^{-3}
rs2764431	1	87578006	A	*HS2ST1*	0.07	2.62 (1.66, 4.13)	3.47×10^{-5}	0.04	0.25
rs34642578	8	17927609	T	*ASAH1*	0.03	4.25 (2.11, 8.55)	4.91×10^{-5}	0.54	7.08×10^{-3}
rs136030	22	46242148	A	*ATXN10*	0.17	1.95 (1.41, 2.68)	4.91×10^{-5}	3.84×10^{-3}	0.02

Definition of abbreviations: Chr = chromosome; CI = confidence interval; GWAS = genome-wide association study; MAF = minor allele frequency; OR = odds ratio; TWAS = transcriptome-wide association study.
The Genome Reference Consortium Human Build 37 (GRCh37) was used for genome reference. The minor allele is the allele modeled as an additive effect.

by Garman and colleagues; *2*) perform a discovery GWAS for pulmonary fibrosis in sarcoidosis (instead of investigating only the AA risk haplotype) in our EA GWAS population to identify shared or distinct genetic risk factors for fibrotic sarcoidosis between AAs and EAs; *3*) test for potential shared genetic risk factors with another fibrosing pulmonary disease, IPF, using a candidate gene approach; and *4*) integrate the association findings with transcriptomic data to prioritize genetic targets via a transcriptome-wide association study (TWAS) for variants associated with fibrotic sarcoidosis.

Using the EA cohort (693 patients with sarcoidosis) from our previous study (5), we compared 156 sarcoidosis cases in Scadding stage IV (fibrotic sarcoidosis) with 537 sarcoidosis with stages I–III. We tested for association between each SNP and fibrotic sarcoidosis using SNPTEST (version 2) (6) adjusted for sex and three principal components. We then compared our results with the four SNPs at *PVT1* identified in the Garman and colleagues study (4). We also tested for association between fibrotic sarcoidosis and 47 SNPs previously identified in IPF studies on the basis of the GWAS catalog (search term: IPF at https://www.ebi.ac.uk/gwas/efotraits/EFO_0000768). A TWAS was performed using PrediXcan (7), which first estimates the expression value of each gene determined by individual-level genotypes using mashr models (generated from GTEx) for lung and whole blood tissues. The next step tests for correlation between imputed gene expression and fibrotic sarcoidosis. Table 1 summarizes the top candidate SNPs with $P_{GWAS} < 5 \times 10^{-5}$ and for which the nearest gene $P_{TWAS} < 0.05$ in either whole blood or lung.

As in Garman and colleagues (4), the SNPs in *PVT1* were rare in our EAs; no individuals had the rare allele. No SNPs reached GWAS significance ($P < 5 \times 10^{-8}$); 401 SNPs had $P < 5 \times 10^{-5}$. The Manhattan plot for the fibrotic sarcoidosis GWAS is shown in Figure 1A. Similarly, none of the SNPs previously identified in IPF studies were significant in our study (threshold $P < 0.05/47 = 0.001$ for 47 SNPs tested). In the TWAS analysis of whole blood, no genes reached the threshold for TWAS significance (threshold $P < 0.05/11{,}813 = 4.23 \times 10^{-6}$ for 11,813 genes tested). The four top genes ($P < 1 \times 10^{-4}$) were *SELENOF* ($P_{TWAS} = 2.00 \times 10^{-5}$), *BFAR* ($P_{TWAS} = 4.09 \times 10^{-5}$), *PARN* ($P_{TWAS} = 4.09 \times 10^{-5}$), and *ZSWIM1* ($P_{TWAS} = 9.88 \times 10^{-5}$) (Figure 1B). Similarly, in lung tissue, no genes reached TWAS significance ($P < 0.05/13{,}769 = 3.63 \times 10^{-6}$ for 13,769 genes tested). Two top genes ($P < 1 \times 10^{-4}$) were *SELENOF* ($P_{TWAS} = 2.00 \times 10^{-5}$) and *CCDC126* ($P_{TWAS} = 3.18 \times 10^{-5}$) (Figure 1C).

Among these genes, we found multiple SNPs (in linkage disequilibrium with each other) in the GWAS across *PARN* had $P < 5 \times 10^{-5}$; the SNP with the smallest GWAS *P* value in *PARN* was rs58103218 ($P_{GWAS} = 2.16 \times 10^{-5}$, $P_{TWAS} = 4.09 \times 10^{-5}$). Rs58103218 was also a significant cis-expression quantitative trait locus for *PARN* (false discovery rate adjusted $P = 1.32 \times 10^{-5}$) and *BFAR* (false discovery rate–adjusted *P* value close to 0) in blood (8).

We used a similar approach to that of Garman and colleagues (4), and, similar to their findings, variants in the *PVT1* gene were nonexistent in our EA subjects. Thus, if they are related to risk of fibrosis in sarcoidosis, much larger EA sample sizes are needed to investigate this potential. We also did not identify shared SNPs between IPF and fibrotic sarcoidosis using the candidate gene approach. However, we identified that *PARN* is a candidate to explore for pulmonary fibrosis in patients with sarcoidosis in our GWAS and TWAS (whole blood) analysis in EA; no other IPF genes, such as *MUC5B*, were associated with fibrotic sarcoidosis (9). Rare variants in *PARN* have been associated with familial pulmonary fibrosis (9) and may be related to shortened telomeres. Among telomere function-related genes, our study identified *PARN* (shortening of telomeres) but not *TERT/TERC* (telomerase activity) as potentially associated with fibrotic sarcoidosis. In summary, our study implied that *PARN*, and therefore telomere shortening, may be a driver for pulmonary fibrosis in a subset of sarcoidosis. *MUC5B* or other well-known IPF genes may not be associated with fibrotic sarcoidosis pathogenesis, consistent with a previous study (10), although much larger sample sizes are needed to better evaluate those associations.

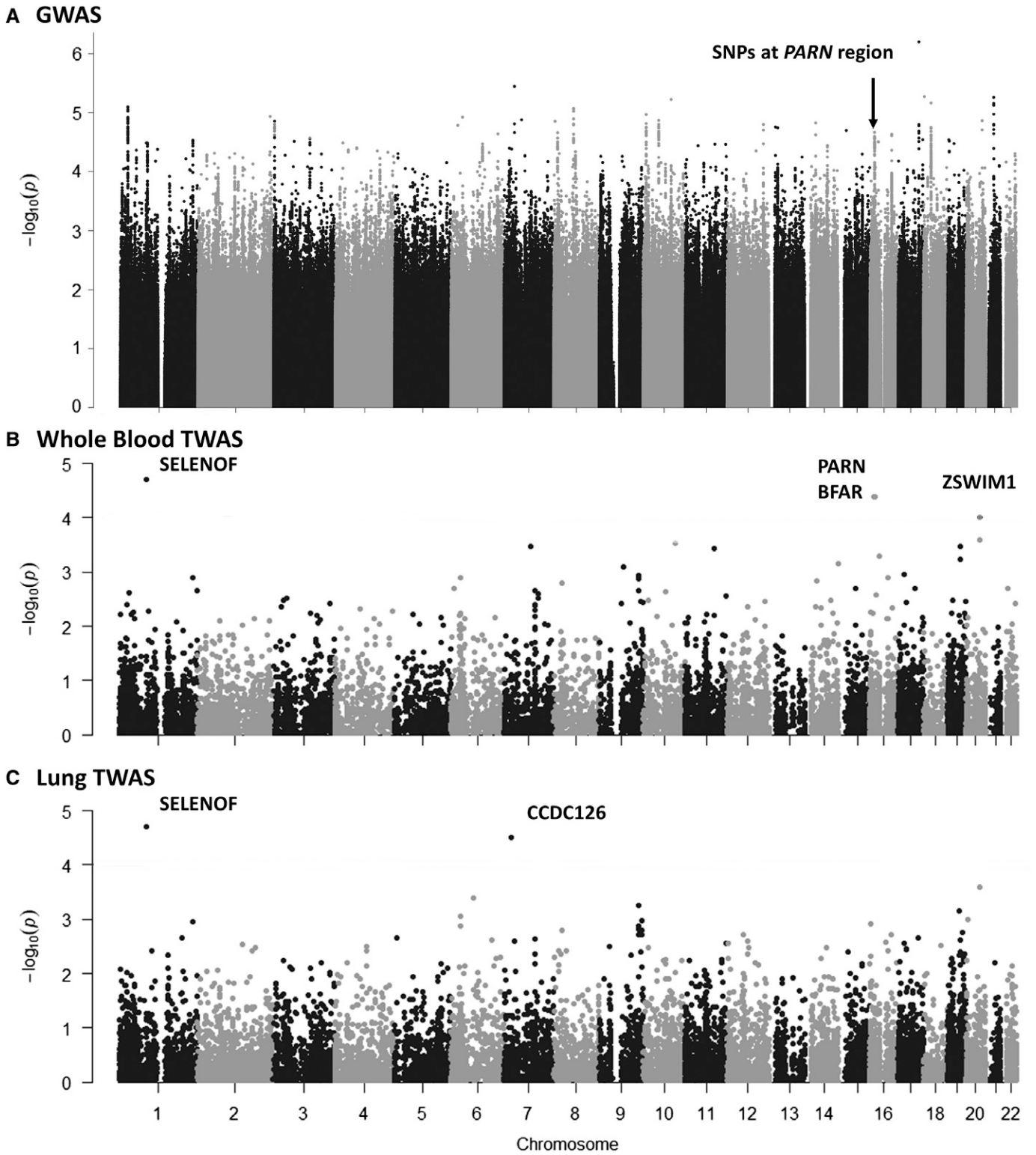

Figure 1. Results of genome-wide association study (GWAS) and transcriptome-wide association study (TWAS) of whole blood and lung. The integrative results showed that *PARN* on chromosome 16 was associated with Scadding's stage IV sarcoidosis (fibrotic sarcoidosis) versus Scadding's stages I, II, and III. (*A*) Manhattan plot of GWAS. (*B*) Manhattan style plot of TWAS of whole blood where each dot represents each single gene. (*C*) Manhattan style plot of TWAS of lung where each dot represents each single gene.

We acknowledge that the power of identifying novel variants of fibrotic sarcoidosis is limited by the small sample size. Therefore, we applied both GWAS and TWAS approaches, which can increase power and point to biologically relevant pathways. Garman and colleagues (4) could not apply this approach using the resource we did, because TWAS databases have relied on a large gene expression database (e.g., GTEx), largely from those with EAs. This again highlights the need for expanded genetic cohorts and biobanks with samples from AAs with and without sarcoidosis. Such resources will be necessary to better understand the etiology and clinical course of sarcoidosis so that we can intervene earlier on the path from healthy lung biology to fibrotic sarcoidosis. ■

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Correspondence and requests for reprints should be addressed to Shu-Yi Liao, M.D., M.P.H., Sc.D, 1400 Jackson Street, Rm G204, Denver, CO 80206. Email: liaos@njhealth.org.

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Metronome-Paced Tachypnea: A Simple, Repeatable Method for Inducing Dynamic Hyperinflation

Christopher B. Cooper[1,2]

[1]Department of Medicine and [2]Department of Physiology, David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, California

ORCID ID: 0000-0002-6314-0903 (C.B.C.).

To the Editor:

Dynamic hyperinflation (DH) reduces inspiratory capacity (IC) and is an important pathophysiological mechanism in obstructive lung diseases (1). DH also causes dyspnea because it increases operational lung volumes, thereby increasing the elastic work of breathing (2). O'Donnell and colleagues (3) showed that, when end-inspiratory lung volume approached within 500 ml of total lung capacity (TLC), patients with chronic obstructive pulmonary disease (COPD) stopped constant-load exercise because of intolerable dyspnea. DH happens when expiratory time is constrained by tachypnea for any reason, including exercise, hypoxemia, and anxiety. It occurs during cycle ergometry exercise in patients with mild COPD (4) and with activities of daily living in patients with moderate to severe COPD (5).

An early study advocated metronome-paced hyperventilation with an approximate doubling of resting breathing frequency as a means of inducing DH (6). The problem with this and other studies that used a similar approach is that the protocol was not rigidly standardized and the stimulus for DH therefore varied from patient to patient. Nevertheless, Lahaije and colleagues (7) showed good overall accuracy in identifying subjects who will experience DH during cardiopulmonary exercise testing (CPET). More recent studies have adopted a standardized protocol using metronome-paced tachypnea (MPT), allowing for tests of validity and reliability as well as enabling the mechanisms of DH to be elucidated (8–10). MPT has been acknowledged as good replacement for CPET in clinical trials, in which the group mean values for DH were not different between CPET and MPT (11).

Methods

The method described here is simple, short in duration, and repeatable (10). The equipment needed is a spirometer that gives volume-versus-time plots and an electronic metronome. Some spirometry equipment has software that is already set up to report IC. While breathing through the flow transducer, resting tidal breathing is monitored. Two repeatable IC measurements within 150 ml should be obtained to establish a baseline value of IC in accordance with American Thoracic Society/European Respiratory Society guidelines (12). Repeatability of IC can be graded similarly to the American Thoracic Society/European Respiratory Society guidelines, with two acceptable measures within 150 ml being grade A/B, two acceptable measures within 200 ml being grade C, and two acceptable measures within 250 ml being grade D.

Several metronome applications are available via the internet (e.g., https://theonlinemetronome.com/). The operator should be able

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