Table 1. Race-Neutral (Global) and Race-Specific References in Relation to Breathlessness and Mortality

Variable	Within each race/ethnicity, %	Breathlessness RRR (95% CI)	Mortality HR (95% CI)
Race/ethnicity and FEV_1 group			
White, no impairment (>LLN_{White})	91.5% of White	*1 (Ref)*	*1 (Ref)*
Black, no impairment (>LLN_{global})	81.6% of Black	1.14 (0.98–1.33)	1.06 (0.86–1.31)
Black, <LLN_{global} but ⩾LLN_{Black}	9.1% of Black	1.57 (1.14–2.17)	2.04 (1.44–2.90)
Black, <LLN_{global} and <LLN_{Black}	9.3% of Black	3.12 (2.32–4.18)	2.52 (1.80–3.51)
White, <LLN_{white} but ⩾LLN_{global}	3.8% of White	3.18 (2.26–4.46)	1.60 (1.02–2.51)
White, <LLN_{global} and <LLN_{White}	4.7% of White	5.42 (3.82–7.69)	5.69 (4.38–7.40)
Body mass index			
<18.5	—	1.39 (0.84–2.29)	1.61 (0.94–2.78)
⩾18.5 to <25	—	*1 (Ref)*	*1 (Ref)*
⩾25 to <30	—	1.14 (0.91–1.44)	1.14 (0.89–1.47)
⩾30	—	2.32 (1.81–2.96)	1.33 (1.04–1.71)

Definition of abbreviations: CI = confidence interval; HR = hazard ratio; LLN = lower limit of normal; RRR = relative-risk ratio.
Adjusted associations with breathlessness using multinomial regression and mortality using Cox proportional-hazards regression. Each estimate is mutually adjusted for all other factors in the table. Participants were grouped by self-reported race/ethnicity and impaired FEV_1, defined as a value less than the lower limit of normal using race-neutral (global) and/or race-specific equations. Data on breathlessness were available and analyzed for people 40+ years. Data were weighted against the U.S. population.

Correspondence and requests for reprints should be addressed to Magnus Ekström, M.D., Ph.D., Department of Medicine, Blekinge Hospital, SE-37185 Karlskrona, Sweden. Email: pmekstrom@gmail.com.

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Inclusivity in Research Matters: Variants in *PVT1* Specific to Persons of African Descent Are Associated with Pulmonary Fibrosis

Lori Garman[1], Nathan Pezant[1], Bryan A. Dawkins[1], Astrid Rasmussen[1], Albert M. Levin[3], Benjamin A. Rybicki[3], Michael C. Iannuzzi[3], Harini Bagavant[2], Umesh S. Deshmukh[2], and Courtney G. Montgomery[1]

[1]Genes and Human Disease and [2]Arthritis and Clinical Immunology, Oklahoma Medical Research Foundation, Oklahoma City, Oklahoma; and [3]Department of Public Health Sciences, Henry Ford Health System, Detroit, Michigan

ORCID IDs: 0000-0001-8868-079X (L.G.); 0000-0002-6993-1669 (A.M.L.).

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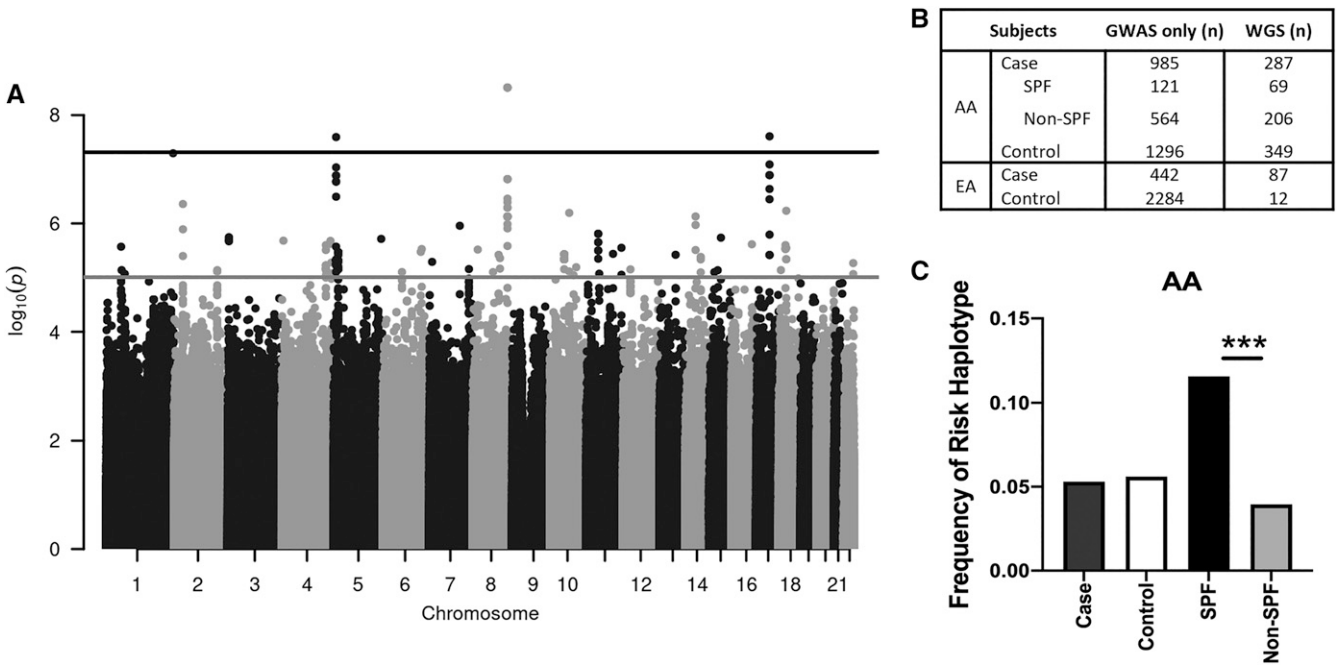
To the Editor:

Sarcoidosis is a systemic, granulomatous disease characterized by a dysregulated immune response. Its leading cause of mortality is pulmonary fibrosis (1). Less than 20% of patients with sarcoidosis develop fibrosis, but risk factors include limited access to care, extrapulmonary involvement, and African ancestry (2, 3). Unfortunately, despite studies highlighting ancestry-specific genetic effects in sarcoidosis and its manifestations (3, 4), genetic studies of sarcoidosis-related pulmonary fibrosis (SPF)—reviewed in Reference (5)—have been limited to patients who are persons of European descent (EUs). Although these studies found associations with genes implicated in fibrosis, inflammation, and immunoregulation, they included heterogeneous patient groups; included only candidate genes; had relatively small sample sizes; and, most important, did not include persons of African descent (AAs), the group most affected by SPF.

To meet the critical need of identifying biomarkers and mechanisms of pulmonary fibrosis in underrepresented populations, we performed the first whole-genome scan (WGS) of SPF in a non-EU population and pulmonary fibrosis in a large cohort of AAs. We identify African-derived risk variants constituting a haplotype that is not present in EUs in plasmacytoma variant translocation 1 (*PVT1*), which encodes for a *PVT1*, long-noncoding RNA associated with inflammation and fibrosis. We further implicate genes involved in profibrotic transforming growth factor beta (TGF-β) and antifibrotic bone morphogenic protein (BMP) signaling that encode well-characterized members of a receptor superfamily that utilizes canonical (SMAD) and noncanonical (phosphatidylinositol-3-kinase/protein kinase B, mitogen-activated protein kinases [MAPK], nuclear factor-κB) signaling pathways. Our findings support a model of SPF in which TGF-β/BMP signaling is dysregulated, ultimately inducing overproduction of collagen and proliferation of fibroblasts in both AAs and EUs. However, the genetic risk factors themselves are ancestry specific and may mediate ancestry-related differences in prognosis and treatment response.

Methods

Our WGS comprised 190 AAs with SPF with confirmed Scadding stage 4 on chest X-ray and 770 AAs with sarcoidosis with Scadding stages 1, 2, and 3 (non-SPF). The variant genotypes were obtained by means of whole-genome sequencing for 295 participants (69 [36%] SPF, 206 [27%] non-SPF) or were imputed from genotype data from ∼1.1 million observed SNPs (Illumina Human Omni1-Quad) using the TOPMed imputation server (Figure 1B). Pre- and postimputation methods were previously described (4) and resulted in 8,405,265 autosomal variants included in single-marker association tests using



	Subjects	GWAS only (n)	WGS (n)
AA	Case	985	287
	SPF	121	69
	Non-SPF	564	206
	Control	1296	349
EA	Case	442	87
	Control	2284	12

Figure 1. Whole-genome scan of persons of African descent (AAs) with sarcoidosis-associated pulmonary fibrosis (SPF) compared with AAs without SPF and frequency of *PVT1* risk variants. (*A*) Manhattan plot highlighting three regions reaching genome-wide statistical significance (upper line; $P < 5 \times 10^{-8}$) at LINC02982 (Chr5, rs11134383; $P = 2.5 \times 10^{-8}$; $MAF_{AA} = 0.072$), *SPOP* (Chr17, 17:49637910; $P = 8.1 \times 10^{-8}$; $MAF_{AA} = 0.068$), and *PVT1* and replicating, at suggestive significance (lower line; $P < 1 \times 10^{-5}$), an effect reported in persons of European descent (EUs) at *TGFB3* (Ch14, rs4252330; $P = 1.2 \times 10^{-6}$; $MAF_{AA} = 0.36$). The four statistically significant variants in *PVT1* (Ch8, rs74730278, rs115311148, rs115529936, and rs115809470; $P < 3.1 \times 10^{-9}$; $MAF_{AA} = 0.047$) are in perfect linkage disequilibrium and form a haplotype with the same frequency as each of the alleles independently. (*B*) The number of individuals in this study with genome-wide genotyping (GWAS) and WGS by disease status. (*C*) The frequency of the *PVT1* risk haplotype in AAs by disease status. The haplotype frequency for cases ($n = 1,271$) included SPF, non-SPF, and those missing Scadding stage but was not statistically significantly different from the frequency excluding those with missing data. ***The difference in the frequency of the risk haplotype in patients with sarcoidosis who have SPF (11.6%) compared with those patients without SPF (non-SPF) (4.0%) was significant at $P = 9.99 \times 10^{-9}$. Note that only one instance of the risk haplotype was found among EUs (haplotypic frequency, 0.018%). GWAS = genome-wide association study; MAF = minor allele frequency; WGS = whole-genome sequencing.

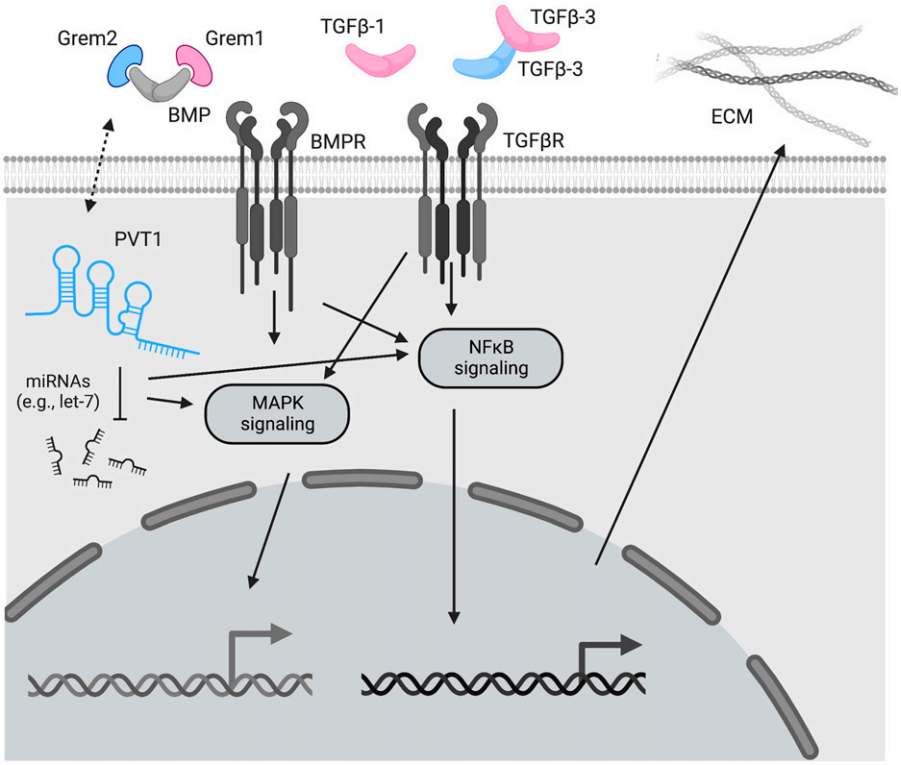


Figure 2. Mechanisms of dysregulation of transforming growth factor β/bone morphogenic protein (TGF-β/BMP) signaling, the master regulator of fibrosis, include ancestry-specific genetic effects. A model, in fibroblasts, of our findings in persons of African descent, compared with those in persons of European descent, of TGF-β/BMP signaling dysregulation at the site of fibrosis formation in the lung. Fibroblasts express receptors for both TGF-β and BMP (TGFβR and BMPR, respectively) as well as PVT1. This model suggests ancestry-specific amplification of profibrotic TGF-β signaling and inhibition of antifibrotic BMP signaling pathways, ultimately leading to transcriptional effects and ECM production characteristic of fibrosis. PVT1, a long noncoding RNA, competes with other endogenous miRNAs, including let-7; promotes fibrosis through collagen production; and acts on the MAPK and NF-κB signaling pathways downstream of TGF-β/BMP signaling. Additional suggestive risk variants in *TGFB3* and the epistatic effect of the BMP antagonist Gremlin-2 implicate multiple modulators of *PVT1*-mediated risk. In comparison, risk variants found in mixed cohorts of patients with progressive and fibrotic sarcoidosis reside in genes encoding TGF-β receptor agonists TGFβ-1 and TGFβ-3 and BMP antagonist Gremlin-1. ECM = extracellular matrix; miRNAs = microRNAs; NF-κB = nuclear factor-κB.

logistic regression in PLINK2, assuming an allelic (multiplicative) genetic model adjusting for sex and the first four ancestry principal components.

For comparison, we calculated haplotype frequencies for AAs with and without SPF—all cases with or without data for Scadding stage (1,273 AAs and 442 EUs)—and control subjects (1,645 AAs, and 2,284 EUs) using whole-genome sequencing, and we imputed data as described earlier. Significant differences in frequencies across groups were assessed using Haploview.

We screened for epistasis between SNPs in *PVT1* and SNPs within 71 genes in the fibrosis pathway by fitting two-way interaction models using FastEpistasis (Bonferroni correction for 5,468 tests; $P < 10^{-5}$) and then estimating interaction effect size for significant pairs of SNPs using PLINK2, adjusting for sex and four principal components.

Results and Discussion

Our WGS revealed three regions exceeding a genome-wide statistical significance of $P < 5 \times 10^{-8}$ (Figure 1A). The most significant region included four variants in *PVT1* in perfect linkage disequilibrium (rs74730278, rs115311148, rs115529936, and rs115809470; each with $P < 3.11 \times 10^{-9}$). The frequency of these variants was virtually identical to the frequency of those in the Allele Frequency Aggregator (https://www.ncbi.nlm.nih.gov/snp/docs/gsr/alfa/). The frequency of the haplotype comprising these variants did not differ between AA cases and controls (5.2% and 5.4%, respectively, $n = 5{,}836$ haplotypes) and was only found once in the EU cohort (5,452 haplotypes; 0.01%), but it was found more frequently in AAs with SPF (11.6%) than in AAs without SPF (4.0%) (odds ratio, 3.28; 95% confidence interval, 1.87–5.78; $P = 9.9 \times 10^{-9}$) (Figure 1C).

PVT1 is an endogenous long noncoding RNA that acts as a sponge for microRNAs such as fibrosis-associated let-7 (6). In animal and cell culture models, PVT1 facilitates collagen production (7), fibroblast proliferation (7), and migration of lung fibroblasts (8). Knockdown or silencing of PVT1 attenuates fibrosis (7) and reduces the expression of major extracellular matrix proteins and their regulators (9). Additionally, PVT1 is proinflammatory by means of the canonically inflammatory MAPK and nuclear factor-κB pathways.

We verified the four associated variants in *PVT1* as expression quantitative trait loci in immune cells in Ensembl (https://useast.ensembl.org/index.html) and QTLbase (http://www.mulinlab.org/qtlbase) and found epistasis between them and six variants within *GREM2* (most significant: rs74511037, $P = 4.3 \times 10^{-6}$; minor

allele frequency in AA = 0.05). We confirmed this association, showing significance at *GREM2* lessened by two orders of magnitude ($P = 4.3 \times 10^{-4}$ to 1.2×10^{-2}) when comparing carriers of the *PVT1* risk haplotype to noncarriers. *GREM2* encodes Gremlin-2, an extracellular BMP antagonist in the same family as Gremlin-1, encoded by *GREM1*, a gene associated with SPF in EUs (5). *GREM2* is more highly expressed in the lung and blood of patients with idiopathic pulmonary fibrosis than in those of control subjects, and in fibrotic compared with nonfibrotic tissue, and elevated Gremlin-2 in human lung fibroblasts increases invasion and migration (10).

Although we acknowledge that our sample size was limited and that we do not have an AA replication cohort, our ability to replicate an effect that was previously identified in EUs within *TGFB3* (5) and to identify novel associations—including known expression quantitative trait loci in an AA-specific haplotype within *PVT1*—in the first WGS of pulmonary fibrosis in AAs highlights the need for and potential impact of research in this area. As in studies of EUs, we found associations implicating dysregulated TGF-β/BMP signaling, but with distinct genetic risk factors. Specifically, for AAs, the SPF risk haplotype in *PVT1* suggests an indirect effect on TGF-β signaling in addition to the effect seen at *TGFB3*, which encodes TGFβ-3, a cytokine involved in fibrosis and immune function (Figure 2). Likewise, our epistasis analysis suggests that SPF in EUs and AAs may uniquely inhibit antifibrotic BMP signaling through Gremlin-1 and Gremlin-2, respectively. Although dysregulation of the TGF-β/BMP signaling pathway may predispose patients to fibrosis, regardless of ancestry, the genetic influences on the mechanism of dysregulation appear to be ancestry specific and may mediate ancestry-related differences in prognosis. As both the first scan of SPF in a non-EU population and the first WGS of pulmonary fibrosis in a large cohort of AAs, our findings highlight the need for inclusion of underrepresented populations in research, as insights into mechanisms and potential treatments may otherwise remain undiscovered. ■

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Correspondence and requests for reprints should be addressed to Courtney G. Montgomery, Ph.D., Genes and Human Disease Research Program, Oklahoma Medical Research Foundation, 825 NE 13th, Research Tower, Suite 2202, Oklahoma City, OK 73104.
Email: courtney-montgomery@omrf.org.

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Outcomes of World Health Organization–defined Severe Respiratory Distress without Shock in Adults in Sub-Saharan Africa

Bickey H. Chang[1], Susan A. Adakun[2], Mary A. Auma[3], Patrick Banura[4], Albert Majwala[5], Amir A. Mbonde[6], Elizabeth Rogawski McQuade[7], Richard Ssekitoleko[8], Mark Conaway[9], and Christopher C. Moore[10]; on behalf of the Severe Respiratory Distress in Africa (SRDA) Investigators

[1]Department of Medicine, University of Minnesota, Minneapolis, Minnesota; [2]Mulago National Referral Hospital, Kampala, Uganda; [3]Department of Medicine, Gulu University, Gulu, Uganda; [4]Ministry of Health Uganda, Kampala, Uganda; [5]Department of Medicine, Lubaga Hospital, Kampala, Uganda; [6]Department of Medicine, Mbarara University of Science and Technology, Mbarara, Uganda; [7]Department of Epidemiology, Emory University, Atlanta, Georgia; [8]World Health Organization, Kampala, Uganda; and [9]Department of Public Health Sciences and [10]Division of Infectious Diseases and International Health, University of Virginia, Charlottesville, Virginia

ORCID ID: 0000-0003-4649-0511 (C.C.M.).

A complete list of SRDA investigators may be found before the beginning of the References.

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