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A Common *NLRC4* Gene Variant Associates With Inflammation and Pulmonary Function in Human Immunodeficiency Virus and Tuberculosis

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Background. Inflammasomes mediate inflammation in adults living with both human immunodeficiency virus (HIV) and tuberculosis (TB), but the relevance of inflammasome gene polymorphisms in TB-associated pulmonary damage is unknown. We hypothesized that functional single-nucleotide polymorphisms (SNPs) in inflammasome pathway genes modify systemic and pulmonary inflammation, contributing to respiratory impairment in adults living with HIV/pulmonary TB.

Methods. This was a prospective cohort study set in South Africa following individuals living with HIV/TB up to 48 weeks post-antiretroviral therapy (ART) initiation. Ten functional SNPs in 5 inflammasome pathway genes were related to circulating inflammatory biomarkers and lung function assessed by spirometry pre- and post-ART initiation. Analyses used 2-sided *t* tests, Wilcoxon rank sum tests, Spearman correlation coefficients, linear regression, and generalized estimating equation models.

Results. Among 102 patients with baseline samples, the minor allele (T) in *NLRC4* rs385076 was independently associated with lower levels of interleukin (IL)-18 and IL-6 before and up to 12 weeks post-ART initiation (Benjamini-Hochberg corrected *P* values < .02). Patients with the CT/TT genotypes also had improved lung function vs CC patients up to 48 weeks post-ART initiation (forced vital capacity, 206 mL higher; 95% confidence interval [CI], 67–345 mL; P = .004 and forced expiratory volume in 1 second, 143 mL higher; 95% CI, 11–274 mL; P = .034).

Conclusions. A common SNP in the *NLRC4* inflammasome may modify TB-associated inflammation in clinically relevant ways. This SNP may identify high-risk groups for lung damage in TB. Inhibition of NLRC4 activity may be an important approach for TB host-directed therapy.

Keywords. inflammasomes; single nucleotide polymorphism; HIV; tuberculosis; lung function.

There were approximately 10 million cases of tuberculosis (TB) in 2017, and TB is 1 of the top 10 causes of death worldwide [1]. TB clinical manifestations and outcomes are linked to tissue inflammation and damage. For example, pulmonary cavitation on chest X ray and lung enhancement on 2-deoxy-2-(fluorine-18) fluoro- D-glucose positron emission tomography–computed tomography are associated with microbiologic treatment failure and impaired pulmonary function [2, 3]. Lung function is an important measure of TB-associated morbidity, as approximately half of those cured of pulmonary disease suffer chronic respiratory impairment [4–6], and pulmonary TB is a major risk factor for chronic obstructive pulmonary disease (COPD) globally [4, 7, 8]. Furthermore, patients who have successfully completed TB treatment have been shown to have reduced quality of life [9], and most of the disability is attributable to chronic pulmonary impairment [10].

Inflammasomes such as NOD-like receptor protein-3 (NLRP3), absent in melanoma 2 (AIM2), and NLR Family CARD domain containing 4 (NLRC4) are part of the innate immune system that recognizes pathogen- and damage-associated molecular patterns [11, 12]. In vitro, *Mycobacterium tuberculosis* (*Mtb*) directly activates NLRP3, the most studied inflammasome. When stimulated, inflammasomes convert pro- interleukin (IL)-1β and pro-IL-18 to their active inflammatory secreted forms [11]. IL-1β and IL-18 have, in turn, been linked to lung tissue destruction in murine models of TB and with decreased lung function in COPD, respectively [13–15]. Furthermore, in

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people living with both human immunodeficiency virus (HIV) and TB, activation of the inflammasome pathway has been linked to the TB immune reconstitution inflammatory syndrome (TB-IRIS) [16, 17], which is characterized by pathologic inflammation during immune restoration while on antiretroviral therapy (ART) [18].

Insights into immunologic mediators of clinically relevant inflammation can be gained via genetic association studies. For example, polymorphisms in inflammasome genes have been associated with altered levels of inflammation and decreased risk of cardiovascular disease deaths in European adults and with early mortality in patients living with both HIV and TB in Botswana [19–21]. We hypothesized that functional variants in inflammasome pathway genes contribute to variation in systemic inflammasome biomarker concentrations and TB-associated pulmonary functional impairment in patients living with both HIV and TB and initiating ART.

METHODS

Study Design and Setting

The Lung Function after TB-IRIS study is a prospective cohort study situated in the Ekurhuleni North District of Gauteng Province, South Africa, to evaluate pulmonary health measures prior to and up to 48 weeks after ART initiation in adults living with both HIV and pulmonary TB. Patients had monthly visits until week 24, after which time they were seen at week 36 and week 48 post-ART initiation. Lung function was evaluated at the baseline (pre-ART) visit and at 4, 12, 24, and 48 weeks post-ART initiation. Phlebotomy for immunologic studies was done at baseline, week 4, and week 12 post-ART.

Study Participants

The study enrolled ART-naive adults living with HIV (aged 18–70 years) with CD4 counts ≤500 cells/μL and a new diagnosis of pulmonary TB as determined using the sputum GeneXpert MTB/RIF assay. Patients were included if they initiated anti-TB therapy <30 days prior to enrollment and were excluded if they were taking immunomodulatory agents, were known or suspected to have drug-resistant TB or TB–meningitis, or were prisoners or were pregnant.

Genotyping Data

Blood was collected in 2.5-mL RNA Paxgene tubes (PreAnalytix, Switzerland) and stored at −80°C. Genomic DNA was extracted using the QIAamp Blood mini DNA extraction kit (Qiagen, Germantown, Maryland). Quantity and quality of DNA were measured on the Nanodrop ND-1000 spectrophotometer (Thermo Fisher Scientific Waltham, Massachusetts). Multiplexed TaqMan genotyping was conducted on the Biomark HD Fluidigm platform using Fluidigm Dynamic arrays as per the manufacturer's instructions (Fluidigm Corporation, San Francisco, CA) [20, 22]. Single-nucleotide polymorphisms (SNPs) in *NLRP3* (rs10925026, rs10754558, rs4612666), *NLRC4* (rs385076), *CARD8* (rs2043211, rs6509365), *IL-18* (rs1946518, rs549908, rs7106524), and *IL-1*β (rs1143627) were determined using validated TaqMan primers and probe sets (Thermo Fisher Scientific, Waltham, Massachusetts) [20]. SNPs were selected based on publications that demonstrated the association with inflammasome biomarker levels and/or with increased risk for inflammatory disease [19, 21, 23–26]. Fluidigm Dynamic Array data were analyzed using the Fluidigm SNP Genotyping Analysis software as previously described [20].

Table 1. Pre-antiretroviral Therapy Characteristics of Patients Living With Human Immunodeficiency Virus and Tuberculosis

Clinical Characteristic	Entire Cohort N = 102	Stratified by *NLRC4* rs385076		
		CC (n = 51)	CT or TT (n = 51)	*P* Value[a]
Male sex, n (%)	58 (56.9)	26 (51)	32 (63)	.23
Age, mean (range in years)	36.8 (20–58)	36 (20–58)	37.5 (22–54)	.45
Antitubercular therapy to antiretroviral therapy initiation interval, median days (IQR); n	24 (15–42); 90	27 (15–47) 45	24 (17–41) 45	.99
Body mass index, median kg/m^2 (IQR)	19.1 (17.9–21.0)	19.1 (17.9–20.6)	19.0 (17.9–21.9)	.84
CD4 count, median cells/μL (IQR); n	113 (49–197); 102	89 (48–180)	135 (57–220)	.22
Viral load, $\log_{10}$ copies/mL (IQR); n	5.19 (4.75–5.86); 102	5.35 (4.5–5.9)	5.1 (4.8–5.7)	.71
Time to culture positivity, median days (IQR); n	14.6 (11.5–17.3); 60	13.6 (11.5–17.1); 31	15.4 (13.4–17.4); 29	.36
Ever smoker, n (%)	41 (40.2)	19 (37.3)	22 (43.0)	.54
FEV_1, mL (IQR)	2.31 (1.86–2.80); 84	2.18 (1.73–2.60); 43	2.52 (2.16–3.01); 41	.053
FEV_1 % predicted	78 (65–88); 84	73 (62–85); 43	80 (61–94); 41	.22
FVC	3.02 (2.46–3.63); 84	2.78 (2.35–3.3); 43	3.3 (2,72-3.74); 41	.01
FVC % predicted	83 (69–92); 84	80 (68–90); 43	84 (72–94); 41	.23
FEV_1/FVC ratio	0.80 (0.73–0.85); 84	0.79 (0.70–0.85); 43	0.81 (0.74–0.85); 41	.65

Abbreviations: FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; IQR, interquartile range.

[a] *P* values are from univariate tests comparing CC vs CT/TT patients.

Systemic Inflammatory Biomarkers

Systemic levels of IL-18 were determined using enzyme-linked immunosorbent assay (ELISA; R&D Systems, Minneapolis, Minnesota) and IL-1β by Luminex (EMD Millipore, Burlington, Massachusetts) as direct readouts of inflammasome activation [11, 27]. IL-6, interferon (IFN)-γ, tumor necrosis factor-α, and IL-10 were measured using Luminex assays (EMD Millipore), as these markers can be downstream targets of the inflammasome pathway [28, 29]. Luminex and ELISAs were conducted per manufacturer's protocols. mRNA levels of these biomarkers were also assessed to evaluate correlations with lung function in an exploratory analysis. Reverse Transcription Master Mix (Fluidigm Corporation, San Francisco, CA) was used for cDNA synthesis using RNA isolated from blood collected in RNA Paxgene tubes, as per manufacturers protocol (PreAnalytix, Switzerland). Next, TaqMan PreAmp Master Mix (2X) (Thermo Fisher Scientific Waltham, Massachusetts) was used for specific target amplification. This was followed by multiplexed quantitative real-time polymerase chain reaction (PCR) using commercially available TaqMan gene expression assays on the Biomark HD Fluidigm platform. Gene expression data were analyzed using Fluidigm's Biomark Real-Time PCR Analysis software. Target gene expression was normalized to *18S* and used to determine the delta-cycle threshold (dCT). Change in gene expression from baseline to week 4 was calculated using the delta–delta CT method and expressed as fold change (2^[dCT at week 4 – dCT at baseline]).

Lung Function

An EasyOne Pro spirometer (New Diagnostic Designs Medical Technologies, Andover, MA) was used to conduct pulmonary function tests (PFTs), which were interpreted according

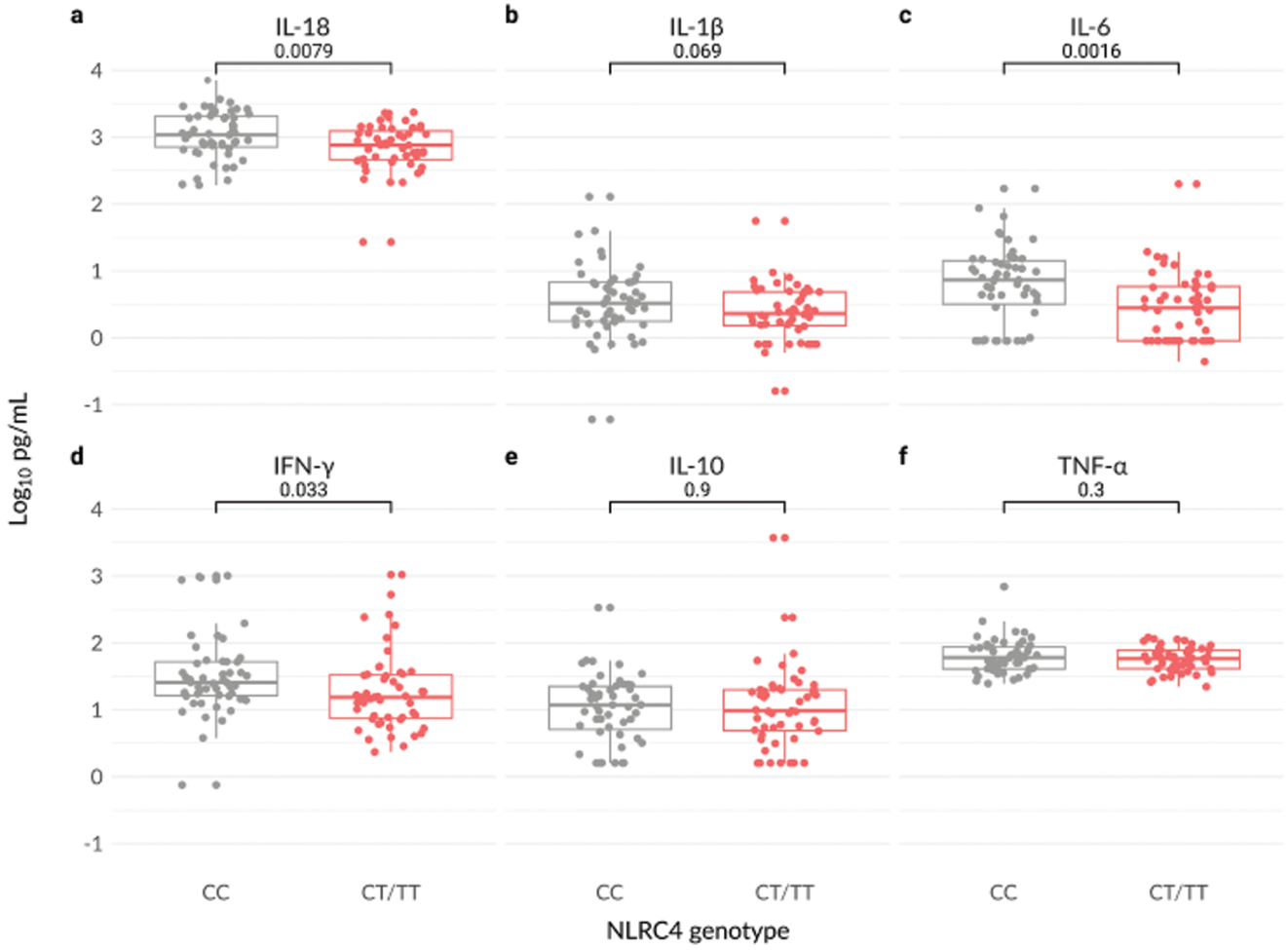

Figure 1. Patients living with both human immunodeficiency virus and tuberculosis who carry the minor allele (T) at the rs385076 locus in *NLRC4* have lower pre-antiretroviral therapy (ART) levels of inflammatory cytokines. Levels of circulating cytokines for (*A*) IL-18, (*B*) IL-1β, (*C*) IL-6, (*D*) IFN-γ, (*E*) IL-10, and (*F*) TNF-α prior to ART initiation were compared between patients with the CC genotype (gray) and those with the CT and TT genotype (pink) at the rs385076 locus in *NLRC4*. Inflammatory markers were measured using plasma from patients and quantitated by Luminex and enzyme-linked immunosorbent assay. Shown are the median and interquartile ranges, as well as the individual values (each dot represents 1 patient) of $\log_{10}$-transformed cytokines. *P* values correspond to uncorrected 2-sample *t* tests comparing inflammatory biomarker levels between the 2 groups. Abbreviations: IFN, interferon; IL, interleukin; pg, picogram; TNF, tumor necrosis factor.

to American Thoracic Society/European Respiratory Society (ATS/ERS) guidelines [30] using reference values for Africans [4, 31]. Forced expiratory volume in 1 second (FEV_1) and forced vital capacity (FVC) obtained from patients undergoing spirometry were obtained as absolute values and as percent-predicted values adjusted for age, height, sex, and race [30]. These lung function measures are associated with survival, and an FEV_1 change of 100 mL has been cited as the minimum clinically important difference in other lung diseases [32, 33]. The quality of PFT data was independently adjudicated by 2 physicians (S. C. A. and G. P. B.) using ATS guidelines. Poor-quality PFTs were excluded from analysis [30].

Statistical Analyses

To be included in the primary analysis of the association between genotype and circulating inflammasome biomarker levels, patients needed to have blood and plasma samples taken at baseline for genotyping and biomarker quantitation, respectively. χ^2 tests were used to determine deviation from Hardy-Weinberg equilibrium. We assumed a dominant model of inheritance comparing patients homozygous and heterozygous for the minor allele (mm + Mm) with those homozygous for the major allele (MM). An additive model of inheritance was considered in exploratory analyses (MM vs Mm vs mm).

Associations between genotype and log_{10}-transformed systemic levels of cytokines were first evaluated using a 2-sided *t* test at baseline. The Benjamini-Hochberg method (false-discovery rate of 0.05) was used to correct for multiple comparisons at this step [34]. SNPs associated with cytokine levels at $P < .05$ in unadjusted analyses were further evaluated for associations with pulmonary function in those with available follow-up data at the time of data analysis. In subsequent univariate analyses, the Wilcoxon rank sum test or 2-sided *t* tests were used, depending on the distribution of the data. In addition, we used linear regression models adjusting for potential confounders including age, sex, smoking, pre-ART CD4 count, and HIV viral load. In these models, factors that changed the unadjusted association by more than 15% were retained in final adjusted models. To gain more clinically relevant insights into lung function throughout TB treatment, we used a generalized estimating equation (GEE) model with an independent correlation structure including all available FEV_1 and FVC data as dependent variables and the SNP of interest as the independent variable among patients with at least 3 longitudinal PFT measures obtained during the 48 weeks of follow-up. Finally, we used Spearman correlation coefficients to explore relationships between cytokine levels and corresponding mRNA levels (with the exception of IL-10) with lung function in order to further evaluate a causal pathway whereby a SNP leads to changes in inflammatory cytokines, which in turn affects lung involvement.

Stata version 14.2 (StataCorp, College Station, TX) was used for analysis, and GraphPad Prism version 7.0c (GraphPad Software Inc, La Jolla, CA) was used for graphics.

Table 2. Associations Between *NLRC4* rs385076-T Genotypes and Log_{10}-Transformed Biomarker Concentrations Prior to Antiretroviral Therapy Initiation Among Patients Living With Both Human Immunodeficiency Virus and Pulmonary Tuberculosis

Inflamatory cytokine	β Coefficient (log_{10} pg/mL)	95% Confidence Interval	R^2	*P* Value
IL-18	−0.18	−.32 to −.05	0.067	.008
IL-1β	−0.17	−.35 to .01	0.056	.067
IL-6	−0.35	−.56 to −.13	0.095	.002
Interferon-γ	−0.25	−.48 to −.02	0.045	.033

β coefficients are from linear regression models and represent the estimated difference in log-transformed biomarker values between groups prior to antiretroviral therapy (ART) initiation with vs without at least 1 T allele at *NLRC4* rs385076 (N = 102). Inclusion of age, sex, smoking, pre-ART CD4 count, and pre-ART human immunodeficiency virus viral load in models did not change unadjusted associations by more than 15% and therefore were not considered confounders in the final models.

Abbreviation: IL, interleukin; pg, picogram.

Table 3. Associations Between *NLRC4* rs385076-T Genotypes and Log_{10}-Transformed Biomarker Concentrations Prior to and Up to 12 Weeks After Antiretroviral Therapy Initiation Among Patients Living With Both Human Immunodeficiency Virus and Pulmonary Tuberculosis

Inflamatory cytokine	β Coefficient (log_{10} pg/mL)	95% Confidence Interval	*P* Value
IL-18	−0.16	−.31 to −.02	.025
IL-1β	−0.15	−.31 to −.00	.056
IL-6	−0.30	−.49 to −.11	.002
Interferon-γ	−0.23	−.45 to −.01	.039

β coefficients are from generalized estimating equation models and represent the estimated difference in log-transformed biomarker values prior to and up to 12 weeks after antiretroviral therapy (ART) initiation between groups with vs without at least 1 T allele at *NLRC4* rs385076 (N = 102). All models considered age, sex, smoking, pre-ART CD4 count, and human immunodeficiency virus viral load as potential confounders. None changed the unadjusted association by 15% or more; therefore, unadjusted models are presented.

Abbreviation: IL, interleukin; pg, picogram.

Ethics

The University of Pennsylvania Institutional Review Board and the University of Witwatersrand Health Research Ethics Committee approved this study. Patients provided written informed consent including for the targeted genotyping.

RESULTS

Baseline Clinical Characteristics

Between July 2016 and March 2018, 102 patients were enrolled and had blood collected prior to ART initiation to determine genotype and inflammatory cytokine levels. Baseline characteristics of patients are shown in Table 1. Fifty-eight patients (57%) were male, the mean age was 37 years (range, 20–58), and the median CD4 count was 113 cells/μL (interquartile range [IQR], 49–197 cells/μL). Forty-one (40%) patients had a history of ever smoking. Eighteen patients (18%) were unable to complete acceptable PFTs at baseline. The median predicted FVC and FEV_1 were 83% and 78%, respectively (Table 1).

Variation in Inflammasome Pathway Genes and Association With Inflammatory Biomarkers

All genotypes were in Hardy-Weinberg equilibrium ($P > .1$; Supplementary Table 1). *NLRC4* rs385076, which has been repeatedly associated with lower IL-18 levels in various populations of healthy adults as well as with a lower risk of cardiovascular death in Europeans [19, 21], was the only variant that was significantly associated with inflammasome-associated biomarkers ($P < .05$; Supplementary Table 2). Patients carrying at least 1 copy of the minor allele (T) at this locus had significantly lower pre-ART levels of IL-18, IL-6, and IFN-γ (Figure 1). The association between pre-ART IL-6 and IL-18 and *NLRC4* rs385076 remained statistically significant ($P = .01$ and $P = .02$, respectively), and there was a borderline association between this SNP and pre-ART IL-1β ($P = .07$) and IFN-γ ($P = .10$) after adjusting for multiple comparisons. There were no significant clinical differences between the CC and CT/TT patients at baseline, although males appeared slightly more likely to have a CT or TT genotype and CC patients had slightly reduced time-to-culture positivity (Table 1). Age, sex, baseline CD4 count, pre-ART HIV viral load, and smoking were not confounders in a linear regression model; therefore unadjusted associations from linear regression are shown in Table 2. In an additive model, each additional *NLRC4* rs385076-T allele tended to be linked to lower baseline IL-18 and IFN-γ levels, although pair-wise comparisons were not generally statistically significant (Supplementary Figure 1). In addition, using all available data at baseline, week 4, and week 12 after ART initiation in a GEE model that evaluated age, sex, CD4 count, smoking, and viral load as potential confounders, having 1 or more T alleles in *NLRC4* rs385076 was significantly associated with lower levels of IL-18, IL-6, and IFN-γ (Table 3). Similar relationships were not observed for mRNA levels (Supplementary Figure 2).

Association Between the *NLRC4* rs385076-T Allele and Lung Function

Patients with at least 1 *NLRC4* rs385076-T allele generally had better absolute FEV_1 values at baseline (median, 2.52 L; IQR,

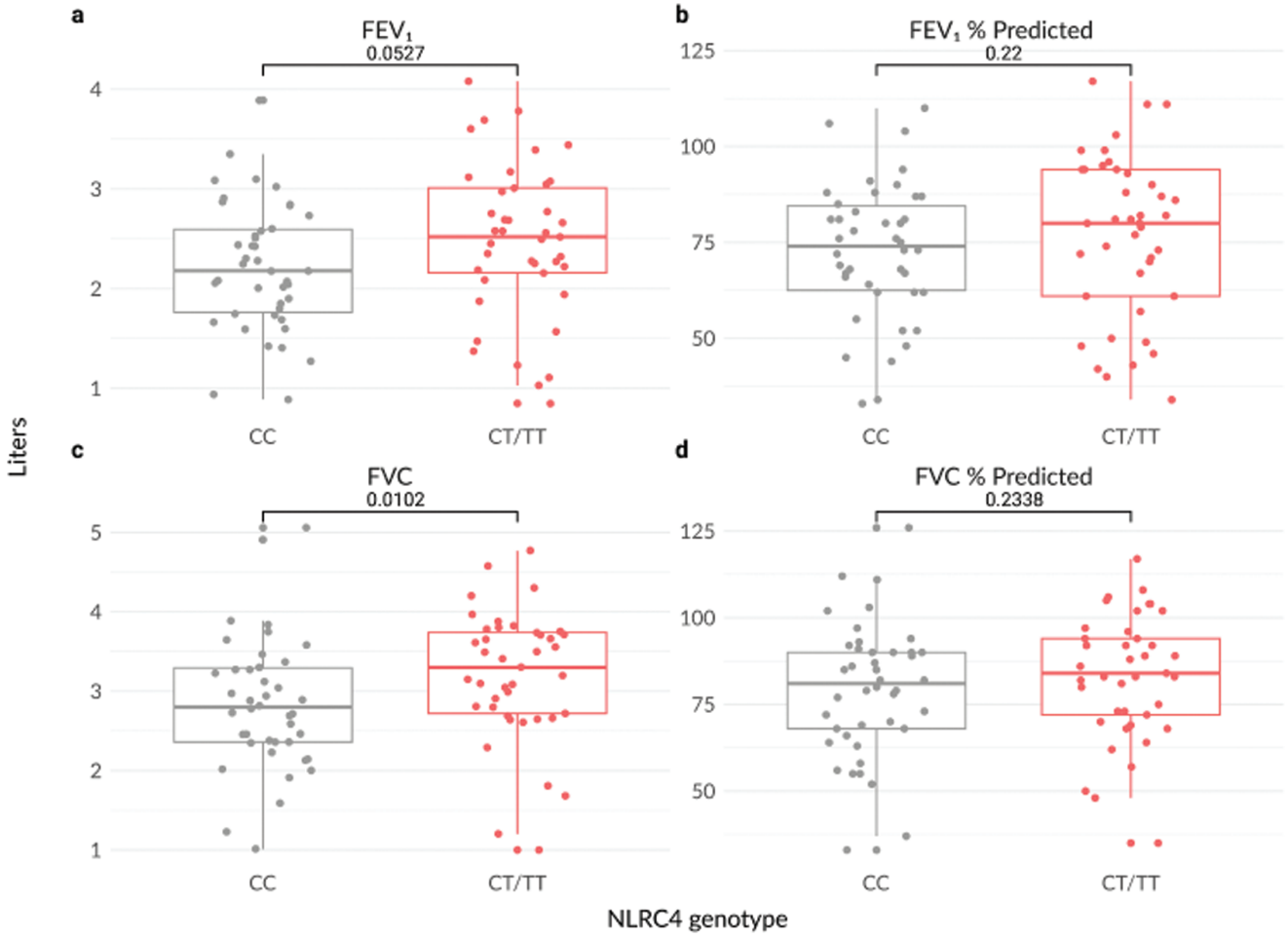

Figure 2. Patients living with both human immunodeficiency virus and tuberculosis who carry the *NLRC4* rs385076-T allele tended to have improved lung function prior to antiretroviral therapy (ART) initiation. Shown are the median and interquartile ranges for FEV_1 in (*A*) liters and (*B*) percent predicted FEV_1, as well as FVC in (*C*) liters and (*D*) percent predicted FVC among patients with the CC vs CT/TT genotype prior to ART initiation. Comparisons were made assuming a dominant model. *P* values shown are from Wilcoxon rank sum tests. Abbreviations: FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity.

2.16–3.01) vs CC patients (median, 2.18 L; IQR, 1.75–2.60; $P = .053$; Figure 2 and Table 1). T allele carriers also had higher FVC values (median, 3.3 L; IQR, 2.72–3.74) compared with CC patients (median, 2.8 L; IQR, 2.36–3.3; $P = .01$) prior to ART initiation (Figure 2 and Table 1). Inclusion of sex, but not pre-ART CD4 count, HIV viral load, age, or smoking history, in the multivariable model decreased the association between the SNP and pre-ART lung function (Table 4). The reduction in the magnitude of the association was likely due, in part, to a greater percent of CT and TT patients being men (who tend to have higher FEV_1 and FVC values). Similarly, the associations were not statistically significant when percent predicted FEV_1 or FVC was used (Figure 2 and Table 1), which take into account sex differences, although the tendency of preserved lung function at baseline in those with 1 or more T alleles persisted. In an additive model, each additional T allele appeared to be associated with improved absolute and predicted FEV_1 and FVC values, although pairwise comparisons were not statistically significant (Supplementary Figure 3).

Next, we compared CC and CT/TT patients using FEV_1 and FVC values over time using all available lung function data up to 48 weeks after ART initiation in patients with at least 3 longitudinal spirometry measurements. In 72 patients with a total of 303 spirometry results during follow-up, CT/TT genotype patients (n = 36) had substantially and significantly higher absolute FVC values (206 mL; 95% confidence interval [CI], 67–345 mL; $P = .004$) during HIV/TB treatment after adjustment for sex, baseline CD4 count, and smoking (Table 5). Adjusted FEV_1 values were also significantly higher (143 mL; 95% CI, 11–274 mL; $P = .034$) in those with CT/TT genotypes (Table 5). Trajectories of lung function over time are shown in Figure 3. Patients included in this analysis were not significantly different from patients not included with respect to clinical characteristics (Supplementary Table 3).

Table 4. Associations Between *NLRC4* rs385076-T Genotypes and Lung Function Prior to Antiretroviral Therapy Initiation Among Patients Living With Both Human Immunodeficiency Virus and Pulmonary Tuberculosis (N = 84)

	β Coefficient (L)	95% Confidence Interval	R^2	*P* Value
Forced expiratory volume in 1 second				
Unadjusted	0.278	−.03 to .58	0.038	.075
Adjusted for sex	0.141	−.13 to .41	0.268	.310
Forced vital capacity				
Unadjusted	0.379	.02 to .73	0.051	.037
Adjusted for sex	0.181	−.11 to .47	0.401	.218

β coefficients are from linear regression models and represent the estimated difference in lung function values between groups prior to antiretroviral therapy (ART) initiation with vs without at least 1 T allele at *NLRC4* rs385076. All models considered age, sex, smoking, pre-ART CD4 count, and human immunodeficiency virus viral load as potential confounders. Only sex changed the unadjusted associations by more than 15%; therefore, only sex is included in the multivariable model.

Table 5. Associations Between *NLRC4* rs385076-T Genotypes and Lung Function Prior to and Up to 48 Weeks After Antiretroviral Therapy Initiation Among Patients Living With Both Human Immunodeficiency Virus and Pulmonary Tuberculosis and With at Least 3 Lung Function Assessments

	β Coefficient (L)	95% Confidence Interval	*P* Value
Forced expiratory volume in 1 second			
Unadjusted	0.249	.096 to .402	.001
Adjusted for sex, baseline CD4 count, and smoking	0.143	.011 to .274	.034
Forced vital capacity			
Unadjusted	0.378	.198 to .559	<.001
Adjusted for sex, baseline CD4 count, and smoking	0.206	.067 to .345	.004

β coefficients are from generalized estimating equation models and represent the estimated difference in spirometry values prior to and up to 48 weeks after antiretroviral therapy (ART) initiation between groups with vs without at least 1 T allele at *NLRC4* rs385076 (N = 72; 303 measurements). All models considered age, sex, smoking, pre-ART CD4 count, and human immunodeficiency virus viral load as potential confounders. Variables that changed the unadjusted association by 15% or more are included in the final models.

Finally, we explored correlations between biomarker levels and lung function at baseline and week 4 of ART. As shown in Supplementary Figure 4, IL-6 cytokine levels, as well as IL-18, IL-6, IFN-γ, and IL-1β mRNA levels, were significantly associated with worse lung function at baseline, week 4 of ART, or both.

DISCUSSION

In this study, an allele (T) at *NLRC4* rs385076 was found to be independently associated with lower systemic levels of IL-18 and IL-6 in South African adults living with active pulmonary TB and HIV who presented to initiate ART. Furthermore, the *NLRC4* rs385076-T allele carriers had, as a group, more preserved pulmonary function throughout TB treatment compared with patients with the CC genotype. Identifying patients at risk for adverse pulmonary outcomes may facilitate novel host-directed therapies designed to improve long-term respiratory status in adults with TB.

The association between *NLRC4* rs385076-T allele carriers and lower levels of IL-18 prior to ART initiation in patients living with both HIV and TB is a novel finding in TB but is consistent with genome-wide association (GWA) studies that have reported a relationship between this SNP and lower IL-18 blood levels in European adults [19, 21]. In our study, we found that approximately half of those who were evaluated had 1 or more copies of the minor protective allele, which extends these results to inflammatory outcomes among individuals living with both HIV and TB. A notable finding from previous work is that while the polymorphism explained <10% of variability in IL-18 levels in various populations, it was associated with a lower risk of cardiovascular disease–related death, suggesting clinical relevance [19, 21]. This is consistent with our results, which indicate

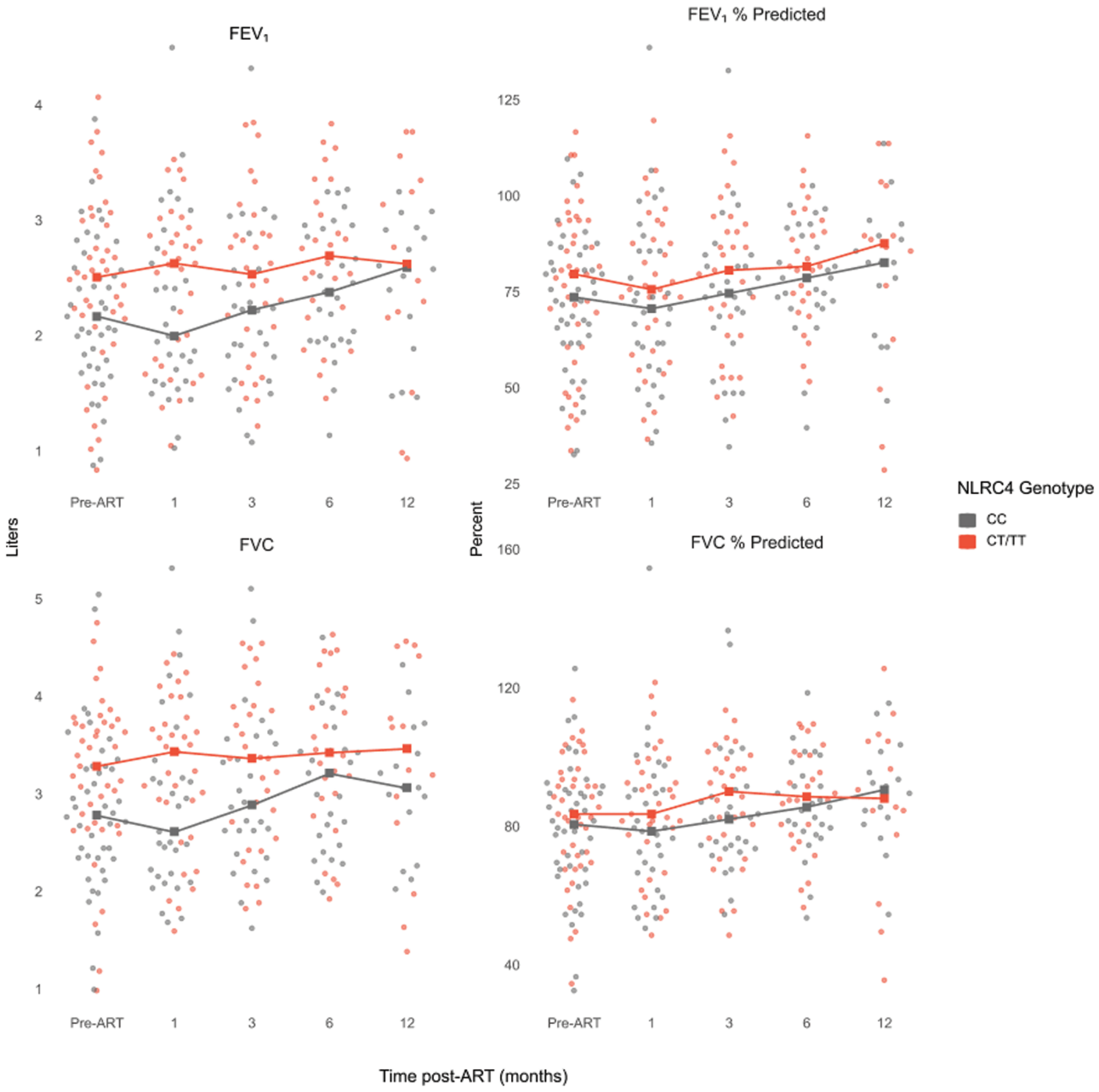

Figure 3. Patients living with both human immunodeficiency virus (HIV) and tuberculosis (TB) who harbor the *NLRC4* rs385076-T allele tended to have elevated lung function compared with those homozygous for the C allele over the course of TB and HIV treatment. Shown are the median and interquartile ranges for (*A*) FEV_1 and (*B*) FVC in liters among patients with the CC (gray) vs CT/TT (pink) genotype at baseline (n = 84), week 4 post-ART (1 month; n = 64), and 3 (n = 60), 6 (n = 57), and 12 months (n = 48) post-ART initiation. Abbreviations: ART, antiretroviral therapy; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity.

that the SNP explains a relatively small amount of circulating biomarker levels (see R^2 values from unadjusted models in Table 2) but is associated with a clinically meaningful metric of respiratory health. While inferring direct causality from SNP association studies is difficult, the *NLRC4* rs385076-T polymorphism is in the 5′untranslated region of exon 2 of the *NLRC4* gene, results in reduced transcription factor binding, and appears to decrease *NLRC4* gene expression [21]. These mechanistic data, combined with our clinical findings, suggest that the rs385076 SNP in *NLRC4* may modify activity of the NLRC4 inflammasome in a manner that is clinically relevant in TB. Moreover, while previous GWA studies [19, 21] did not look at the association between *NLRC4* rs385076-T and IL-6 and IFN-γ levels, our data suggest that this SNP may also modulate proinflammatory cytokines downstream of IL-18 activation [28, 29].

Our results indicate that the role of the NLRC4 inflammasome needs further consideration in understanding TB-associated

inflammation and pulmonary damage, for example, in the development of host-directed therapies that aim to decrease inflammatory tissue destruction. Broad inflammasome inhibition, for example, via use of an anti–IL-1β antibody has been shown to have beneficial clinical effects by decreasing the risk of cardiovascular disease–related death in at-risk adults [35]. In the same trial, however, inflammasome inhibition was associated with an increased risk of fatal infections, which has spurred efforts to inhibit specific inflammasome types that are associated with inflammation in specific diseases [36]. In this sense, our results are notable because in vitro and in vivo studies have concluded that IL-18 and IL-1β production after *Mtb* infection is mediated not by *NLRC4* but specifically by *NLRP3* [37, 38]. Highlighting a possible role for the NLRC4 inflammasome in TB inflammation is therefore important in that it may provide a novel direction for host-directed therapies in this globally relevant disease. While additional studies are needed, our findings that relate biomarker levels and their corresponding mRNA levels to pulmonary function in patients with TB lend plausibility to the pathway whereby the *NLRC4* SNP evaluated here may affect lung damage in TB. Consistent with our data, IL-18 and IL-6, as well as genetic polymorphisms that contribute to variability in these biomarkers, have been implicated in COPD-associated lung dysfunction [13–15, 39].

Our sample size limited our ability to detect some apparently substantial effects on lung function with statistical significance and to examine confounding from population stratification and ethnicity. In addition, while all analyses suggested lower biomarker concentrations in those with 1 or more T alleles, results for IFN-γ and IL-1β were of borderline significance after adjusting for multiple comparisons. Thus, the associations with IL-6 and IL-18, which were significant after *P* value correction and after assessment for confounding, can be considered the most robust. In addition, associations between the *NLRC4* polymorphism and lung function metrics were not significant prior to ART initiation after adjustment (or when expressed as percent predicted values, which take into account sex). While confounding by sex was apparent at baseline, adjusted longitudinal models that take into account sex supported the association between the T allele and more preserved lung function for up to 48 weeks after ART initiation (Table 5). Furthermore, the associations between, for example, FVC and the *NLRC4* polymorphism over time were beyond the magnitude that ATS/ERS considers to be clinically important (ie, 100 mL in FEV_1) [32, 33]. Another limitation is the fact that our study focused on patients living with both HIV and TB. Whether these findings persist years after treatment completion and whether the results can be generalized to patients living with TB or HIV alone needs further evaluation, as suggested by recent data linking *NLRC4* activation to HIV progression [40].

In conclusion, we found that a SNP in NLRC4 is associated with lower lung inflammation and pulmonary impairment in patients living with both HIV and TB and initiating ART in South Africa. Future studies to evaluate the role of this inflammasome type in TB-associated inflammation and tissue damage and trials to evaluate inflammasome inhibition to decrease TB-associated morbidity are needed.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author contributions. S. R. conceptualized and designed the genetic substudy; performed data collection, analysis, and initial interpretation; and drafted the manuscript. S. C. A. and G. P. B. performed analysis of lung function data. P. M., M. M., I. N., and M. dT. V. coordinated patient enrollment as well as clinical data collection. W. C. contributed to data analysis. G. P. B., H. K., G. C., R. S. W., S. R., and D. W. conceptualized and designed the parent study as well as provided guidance on the conduct of the substudy. G. P. B., C. T. T., and D. W. mentored S. R. and facilitated the conduct of this study. S. R. and G. P. B. wrote the initial drafts of the manuscript. All authors contributed to the writing of the manuscript and approved the final draft.

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