Online Data Supplement

Rheumatoid Arthritis-Associated Autoantibodies and Subclinical Interstitial Lung Disease: The Multi-Ethnic Study of Atherosclerosis

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Participants and data sources

In the current study, we included participants from the MESA study (n=6,814) as well as from two NHLBI-funded MESA ancillary studies: the MESA Lung Substudy and the MESA Autoimmunity Substudy. In 2004, the MESA Lung Substudy randomly sampled 3,965 MESA participants who had consented to genetic analyses, underwent baseline measures of endothelial function, and attended MESA Exam 3 or 4. Of these MESA Lung participants, 3,856 underwent spirometry and additional questionnaires at Exam 3 or 4 (2004-2006) and 2,727 underwent full lung CT scans at Exam 5 (2010-2012). An additional 410 MESA participants were recruited in 2010-2012 to undergo full lung CT scans as well. Of these 3,137 full lung CT scans, 195 were not read for ILA and 35 had unreadable scans, leaving 2,907 valid ILA measurements. As has been done in previous studies of ILA,[1-3] we excluded 477 full lung CT scans that we read as "indeterminate" for ILA, leaving 2,430 full lung CT scans for analysis. Of the 2,430 participants with full lung CT scans for analysis, RF IgM and RF IgA were measured in 2,403 at Exam 1 and anti-CCP was measured in 2,399.

Measurement of exposure

A positive RF was defined as >25 U/mL for IgM and >35 U/mL for IgA. A positive anti-CCP was defined as a level of >5 U/mL.

Quantitative CT Attenuation on Cardiac CT Scan

Measurements of CT attenuation were quantified on the lung fields of non-contrast cardiac CTs, which image about 70% of the lung volume from the carina to the bases.[4] Each participant underwent 2 sequential scans at full inspiration. The scan with the greater volume of

air was used for analysis, unless the scans were discordant in quality (as determined by the scan quality control score), in which case the higher-quality scan was used.[4]

Interstitial Lung Abnormalities on Chest CT

Full non-contrast lung CT scans were performed at suspended full inspiration on 64-slice scanners (GE and Siemens) using the MESA-Lung/SPIROMICS protocol.[5] Images were reconstructed using 0.625 mm slice thickness. During a training period, 5 expert thoracic radiologists (J.H.M.A, J.D.N., P.H.N., S.K.S., J.R.W.) independently reviewed 20 randomly selected MESA exam 5 full lung CT scans and scored the presence or absence of each type of ILA (ground glass abnormalities, reticular abnormalities, diffuse centrilobular nodularity, nonemphysematous cysts, honeycombing, traction bronchiectasis) using a case-report form.[2, 6, 7] All 20 scans were then reviewed as a group via teleconference. Each exam 5 full lung CT scan was then assigned to 1 of the 5 expert radiologists for a formal ILA read.

Statistical analysis

In order to calculate the p-value for trend across quartiles of autoantibody levels, we created a 4-level categorical variable corresponding to the four quartiles. The value assigned to this variable was equal to the median value within each quartile. For example, the median value of RF IgM within the first RF IgM quartile was 3 U/mL, so all RF IgM values in the first quartile were assigned a value of 3. We included this 4-level variable as a continuous independent variable in both unadjusted and fully adjusted models (without any other autoantibody variable). We used the Wald p-value for this variable as the p-value for trend across quartiles

We used the *mi impute* command in Stata to perform multiple imputation by chained equations to account for missing covariate data. This method accommodates arbitrary patterns of missing data and uses a Gibb's-like algorithm to sequentially impute multiple variables using univariate fully conditional specifications.[8] Within the imputation model, we used linear regression for continuous variables, multinomial logistic regression for nominal variables, and logistic regression for binary variables. There were 5 imputed datasets. Only 0.4% of participants had any missing data.

REFERENCES

1. Podolanczuk AJ, Oelsner EC, Barr RG, et al. High attenuation areas on chest CT in community-dwelling adults: the MESA study. *Eur Respir J* (in press).

2. Washko GR, Hunninghake GM, Fernandez IE, et al. Lung volumes and emphysema in smokers with interstitial lung abnormalities. *N Engl J Med* 2011;364:897-906.

3. Doyle TJ, Washko GR, Fernandez IE, et al. Interstitial lung abnormalities and reduced exercise capacity. *Am J Respir Crit Care Med* 2012;185:756-62.

4. Hoffman EA, Jiang R, Baumhauer H, et al. Reproducibility and validity of lung density measures from cardiac CT Scans--The Multi-Ethnic Study of Atherosclerosis (MESA) Lung Study. *Acad Radiol* 2009;16:689-99.

5. Couper D, LaVange LM, Han M, et al. Design of the Subpopulations and Intermediate Outcomes in COPD Study (SPIROMICS). Thorax 2014;69:491-4.

6. Hunninghake GM, Hatabu H, Okajima Y, et al. MUC5B promoter polymorphism and interstitial lung abnormalities. *N Engl J Med* 2013;368:2192-200.

7. Washko GR, Lynch DA, Matsuoka S, et al. Identification of early interstitial lung disease in smokers from the COPDGene Study. *Acad Radiol* 2010;17:48-53.

8. StataCorp. STATA Multiple Imputation Reference Manual: Release 13. 2013 [cited 2016 July 8]; 13:[Available from: https://www.stata.com/manuals13/mi.pdf]

Supplemental Table 1: Baseline Characteristics by IgA Rheumatoid Factor Quartile

	IgA Rheumatoid Factor Quartile			
	< 6.04 U/mL	6.04 to 9 U/mL	9.04 to 15.86 U/mL	>15.86 U/mL
No. of subjects	1969	1554	1529	1684
Demographics & anthropometrics				
Age, years	60 (52-68)	62 (53-70)	63 (54-71)	65 (56-72)
Female sex	1122 (57.0)	860 (55.3)	738 (48.3)	843 (50.1)
Race/ethnicity				
White	1075 (54.6)	581 (37.4)	463 (30.3)	480 (28.5)
African American	389 (19.8)	401 (25.8)	463 (30.3)	597 (35.5)
Chinese American	184 (9.3)	246 (15.8)	199 (13.0)	174 (10.3)
Hispanic	321 (16.3)	326 (21.0)	404 (26.4)	433 (25.7)
Height, cm	166.8 ± 9.8	165.8 ± 9.9	166.5 ± 10.4	166.2 ± 10.1
Weight, lbs	172.1 ± 37.9	171.4 ± 39.4	173.6 ± 38.2	176.0 ± 37.3
Body mass index, kg/m^2	28.0 ± 5.4	28.2 ± 5.6	28.3 ± 5.4	28.9 ± 5.5
Body mass index category, kg/m^2				
Underweight	12 (0.6)	19 (1.2)	12 (0.8)	13 (0.8)
Normal	613 (31.1)	467 (30.1)	407 (26.6)	392 (23.3)
Overweight	763 (38.8)	568 (36.6)	617 (40.4)	690 (41.0)
Obese	581 (29.5)	500 (32.2)	493 (32.2)	589 (35.0)
Waist circumference, cm	97.1 ± 14.5	97.6 ± 14.7	98.3 ± 13.9	99.7 ± 14.4
Hip circumference, cm	105.5 ± 11.1	105.5 ± 11.9	105.1 ± 11.2	106.3 ± 11.7
Smoking				
Never smoker	930 (47.2)	742 (47.8)	698 (45.7)	681 (40.5)
Former smoker	794 (40.3)	590 (38.0)	604 (39.5)	743 (44.2)
Current smoker	245 (12.4)	220 (14.2)	226 (14.8)	259 (15.4)
Cigarette pack-years (among ever-smokers)	13.6 (3.0-30.0)	14.0 (3.0-32.0)	14.5 (3.5-33.0)	15.8 (3.8-34.5)
Spirometry				

FEV1, L	2.5 ± 0.7	2.3 ± 0.7	2.4 ± 0.7	2.3 ± 0.7
FEV1, %predicted	93.9 ± 16.5	94.2 ± 18.6	94.1 ± 18.1	92.9 ± 19.5
FVC, L	3.3 ± 1.0	3.1 ± 1.0	3.2 ± 1.0	3.1 ± 0.9
FVC, %predicted	95.4 ± 15.4	95.6 ± 16.8	95.4 ± 16.3	95.2 ± 16.9
FEV1/FVC ratio	0.75 ± 0.08	0.75 ± 0.08	0.75 ± 0.08	0.74 ± 0.10
Computed tomography				
Total imaged lung volume (gas + tissue), cm^3	2813.0 ± 761.2	2735.8 ± 828.3	2771.5 ± 820.3	2745.1 ± 815.8
Emphysema, %	3.1 (1.3-6.1)	2.8 (1.2-5.7)	2.8 (1.2-5.6)	2.7 (1.1-5.2)
Respiratory diseases				
Self-reported emphysema, %	20 (1.0)	21 (1.4)	34 (2.2)	27 (1.6)
Self-reported asthma, %	199 (10.1)	154 (9.9)	144 (9.4)	164 (9.7)
Hepatitis C	8 (0.4)	7 (0.5)	9 (0.6)	23 (1.4)

Data presented as mean (SD), median (IQR), and frequency (percentage)

Missing data are as follows: Waist circumference (n = 1); Hip circumference (n = 1); Smoking (n = 4); FEV1 (n = 2,925); FVC (n = 2,939); FEV1/FVC (n = 2942); Self-reported emphysema (n = 1); Self-reported asthma (n = 2); Hepatitis C (n = 1)

Supplemental Table 2: Baseline Characteristics by Anti-Cyclic Citrullinated Peptide Antibody Quartile

	Anti-Cyclic Citrullinated Peptide Antibody Quartile			
	< 0.17 U/mL	0.17 to 0.2 U/mL	0.21 to 0.5 U/mL	> 0.5 U/mL
No. of subjects	1706	1696	1711	1615
Demographics & anthropometrics				
Age, years	62 (54-70)	62 (53-70)	62 (53-70)	63 (54-71)
Female sex	918 (53.8)	884 (52.1)	896 (52.4)	858 (53.1)
Race/ethnicity				
White	736 (43.1)	588 (34.7)	584 (34.1)	684 (42.4)
African American	327 (19.2)	423 (24.9)	530 (31.0)	569 (35.2)
Chinese American	199 (11.7)	255 (15.0)	226 (13.2)	123 (7.6)
Hispanic	444 (26.0)	430 (25.4)	371 (21.7)	239 (14.8)
Height, cm	165.8 ± 10.0	166.0 ± 10.1	166.5 ± 10.0	167.1 ± 10.0
Weight, lbs	172.4 ± 37.5	172.5 ± 38.6	173.3 ± 38.1	175.1 ± 38.6
Body mass index, kg/m^2	28.4 ± 5.4	28.3 ± 5.5	28.3 ± 5.5	28.4 ± 5.4
Body mass index category, kg/m^2				
Underweight	18 (1.1)	9 (0.5)	15 (0.9)	14 (0.9)
Normal	456 (26.7)	485 (28.6)	493 (28.8)	442 (27.4)
Overweight	682 (40.0)	664 (39.2)	653 (38.2)	639 (39.6)
Obese	550 (32.2)	538 (31.7)	550 (32.1)	520 (32.2)
Waist circumference, cm	98.2 ± 14.3	98.2 ± 14.5	97.9 ± 14.2	98.3 ± 14.7
Hip circumference, cm	105.7 ± 11.6	105.2 ± 11.4	105.5 ± 11.5	105.9 ± 11.4
Smoking				
Never smoker	747 (43.8)	782 (46.1)	816 (47.8)	701 (43.4)
Former smoker	701 (41.1)	686 (40.5)	654 (38.3)	688 (42.6)
Current smoker	258 (15.1)	227 (13.4)	239 (14.0)	225 (13.9)

Cigarette pack-years (among ever-smokers)	13.6 (3.0-31.3)	13.5 (2.6-31.0)	15.0 (3.2-34.0)	16.0 (4.5-35.0)
Spirometry				
FEV1, L	2.4 ± 0.7	2.4 ± 0.7	2.4 ± 0.7	2.3 ± 0.7
FEV1, %predicted	93.9 ± 17.6	95.0 ± 18.1	94.0 ± 18.2	92.0 ± 18.4
FVC, L	3.2 ± 1.0	3.2 ± 1.0	3.2 ± 0.9	3.1 ± 0.9
FVC, %predicted	95.7 ± 15.5	96.2 ± 16.8	95.6 ± 16.5	94.1 ± 16.2
FEV1/FVC ratio	0.75 ± 0.08	0.75 ± 0.08	0.75 ± 0.09	0.74 ± 0.09
Computed tomography				
Total imaged lung volume (gas + tissue), cm^3	2800.4 ± 791.3	2769.0 ± 842.8	2737.0 ± 775.3	2770.8 ± 810.5
Emphysema, %	2.7 (1.2-5.2)	2.7 (1.1-5.3)	2.7 (1.2-5.6)	3.5 (1.4-6.7)
Respiratory diseases				
Self-reported emphysema, %	20 (1.2)	30 (1.8)	29 (1.7)	23 (1.4)
Self-reported asthma, %	171 (10.0)	153 (9.0)	173 (10.1)	162 (10.0)
Hepatitis C	6 (0.4)	9 (0.5)	16 (0.9)	16 (1.0)

Data presented as mean (SD), median (IQR), and frequency (percentage)

Missing data are as follows: Waist circumference (n = 1); Hip circumference (n = 1); Smoking (n = 4); FEV1 (n = 2,921); FVC (n = 2,935); FEV1/FVC (n = 2938); Self-reported emphysema (n = 1); Self-reported asthma (n = 2); Hepatitis C (n = 1)

Supplemental Table 3: P-values for the Linearity of the Associations of Autoantibodies with HAA and ILA

	HAA			ILA		
	RF IgM	RF IgA	Anti-CCP	RF IgM	RF IgA	Anti-CCP
All participants	0.68	0.06	0.07	0.28	0.27	0.04
Ever smokers	0.52	0.27	0.29	0.25	0.28	0.25
Never smokers	0.52	0.15	0.26	0.85	0.36	0.21

Supplemental Table 4: Stratified Analyses for Association of RF IgM with High Attenuation Areas and Interstitial Lung Abnormalities on CT among Ever Smokers

	Adjusted relative percent difference (95% CI) in HAA per doubling of RF IgM*	p-value for interaction	Adjusted prevalence ratio (95% CI) for ILA per doubling of RF IgM†	p-value for interaction
Age continuous		0.15		0.14
Age decade at exam 1		0.44		0.25
44-54	1.48 (0.53 to 2.43)		1.16 (0.95 to 1.41)	
55-64	0.44 (-0.52 to 1.41)		1.29 (1.11 to 1.48)	
65-74	0.73 (-0.09 to 1.55)		1.05 (0.91 to 1.21)	
75-84	0.27 (-0.99 to 1.55)		1.08 (0.94 to 1.25)	
Sex		0.09		0.69
Female	1.29 (0.53 to 2.06)		1.15 (1.02 to 1.29)	
Male	0.51 (-0.11 to 1.13)		1.13 (0.99 to 1.28)	
Race		0.81		0.19
White	0.41 (-0.23 to 1.05)		1.22 (1.06 to 1.40)	
African American	0.97 (0.12 to 1.84)		1.18 (1.03 to 1.35)	
Chinese American	-0.01 (-2.01 to 2.03)		0.59 (0.37 to 0.94)	
Hispanic	1.14 (-0.06 to 2.35)		1.03 (0.87 to 1.22)	
Race		0.64		
African American	0.97 (0.12 to 1.84)		1.18 (1.03 to 1.35)	0.56
Other	0.79 (0.21 to 1.37)		1.11 (1.001 to 1.23)	
Pack-years		0.21		0.81
BMI continuous		0.72		
BMI categories		0.63		
Underweight	-7.75 (-11.25 to -4.10)			
Normal	0.30 (-0.63 to 1.23)			
Overweight	0.90 (0.11 to 1.69)			
Obese	1.04 (0.23 to 1.85)			
Hepatitis C		0.41		
Negative	0.76 (0.27 to 1.26)		1.14 (1.05 to 1.25)	
Positive	2.26 (-3.15 to 7.98)		**	

* Adjusted for age, sex, race, study site, BMI, waist circumference, total volume of imaged lung, percent emphysema, and tube current

† Adjusted for age, sex, and race

** Only 12 participants who are hepatitis C positive and ever smokers had ILA reads.

Stratified analyses and tests for interaction were performed on complete cases.

Supplemental Table 5: Stratified Analyses for Association of RF IgA with High Attenuation Areas and Interstitial Lung Abnormalities on CT among Ever Smokers

	Adjusted relative percent difference (95% CI) in HAA per doubling of RF IgA*	p-value for interaction	Adjusted prevalence ratio (95% CI) for ILA per doubling of RF IgA†	p-value for interaction
Age continuous		0.61		0.48
Age decade at exam 1		0.50		0.33
44-54	1.43 (0.23 to 2.65)		1.03 (0.75 to 1.41)	
55-64	0.65 (-0.46 to 1.77)		1.19 (0.97 to 1.45)	
65-74	1.47 (0.53 to 2.41)		1.18 (1.08 to 1.29)	
75-84	1.59 (0.03 to 3.18)		0.95 (0.80 to 1.13)	
Sex		0.24		0.79
Female	1.72 (0.83 to 2.61)		1.10 (0.98 to 1.23)	
Male	0.99 (0.24 to 1.75)		1.16 (1.03 to 1.31)	
Race		0.38		0.053
White	1.14 (0.42 to 1.87)		1.28 (1.15 to 1.42)	
African American	1.55 (0.49 to 2.63)		1.10 (0.95 to 1.27)	
Chinese American	-1.83 (-4.40 to 0.81)		0.52 (0.43 to 0.63)	
Hispanic	1.47 (0.05 to 2.91)		1.08 (0.89 to 1.32)	
Race		0.73		0.52
African American	1.55 (0.49 to 2.63)		1.10 (0.95 to 1.27)	
Other	1.34 (0.67 to 2.01)		1.16 (1.05 to 1.27)	
Pack-years		0.81		0.21
BMI continuous		0.26		
BMI categories		0.31		
Underweight	-6.36 (-12.70 to 0.44)			
Normal	0.46 (-0.67 to 1.60)			
Overweight	1.39 (0.48 to 2.30)			
Obese	1.82 (0.85 to 2.80)			
Hepatitis C		0.37		
Negative	1.29 (0.71 to 1.87)		1.14 (1.05 to 1.24)	
Positive	2.12 (-6.57 to 11.62)		**	

* Adjusted for age, sex, race, study site, BMI, waist circumference, total volume of imaged lung, percent emphysema, and tube current

† Adjusted for age, sex, and race

** Only 12 participants who are hepatitis C positive and ever smokers had ILA reads.

Stratified analyses and tests for interaction were performed on complete cases.

Supplemental Table 6: Stratified Analyses for Association of Anti-CCP with High Attenuation Areas and Interstitial Lung Abnormalities on CT among Ever Smokers

	Adjusted relative percent difference (95% CI) in HAA per doubling of anti-CCP*	p-value for interaction	Adjusted prevalence ratio (95% CI) for ILA per doubling of anti-CCP†	p-value for interaction
Age continuous		0.35		0.44
Age decade at exam 1		0.16		0.50
44-54	1.75 (-0.33 to 3.88)		0.75 (0.37 to 1.51)	
55-64	-0.24 (-2.00 to 1.55)		1.26 (1.11 to 1.44)	
65-74	1.42 (0.09 to 2.77)		1.09 (0.90 to 1.31)	
75-84	2.67 (0.55 to 4.83)		1.02 (0.90 to 1.15)	
Sex		0.41		0.78
Female	1.03 (-0.20 to 2.28)		1.08 (0.93 to 1.27)	
Male	1.71 (0.49 to 2.94)		1.15 (0.98 to 1.34)	
Race		0.03		0.19
White	0.12 (-0.97 to 1.22)		1.23 (1.13 to 1.33)	
African American	0.92 (-0.64 to 2.49)		0.92 (0.42 to 2.05)	
Chinese American	3.66 (-0.69 to 8.20)		0.04 (0.001 to 1.33)	
Hispanic	3.85 (1.57 to 6.18)		1.15 (1.01 to 1.31)	
Race		0.54		0.20
African American	0.92 (-0.64 to 2.49)		0.92 (0.42 to 2.05)	
Other	1.62 (0.58 to 2.67)		1.18 (1.09 to 1.27)	
Race		0.004		0.85
Hispanic	3.85 (1.57 to 6.18)		1.15 (1.01 to 1.31)	
Other	0.58 (-0.32 to 1.48)		1.12 (0.96 to 1.30)	
Pack-years		0.02		0.55
BMI continuous		0.85		
BMI categories		0.99		
Underweight	0.58 (-11.09 to 13.78)			
Normal	1.68 (0.01 to 3.37)			
Overweight	1.30 (0.02 to 2.61)			
Obese	0.89 (-0.73 to 2.54)			
Hepatitis C		0.08		
Negative	1.17 (0.30 to 2.05)		1.13 (1.02 to 1.24)	
Positive	1.07 (-12.62 to 16.91)		**	

* Adjusted for age, sex, race, study site, BMI, waist circumference, total volume of imaged lung, percent emphysema, and tube current

† Adjusted for age, sex, and race

** Only 8 participants who are hepatitis C positive and ever smokers had ILA reads.

Stratified analyses and tests for interaction were performed on complete cases.