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Annexin A11 is associated with pulmonary fibrosis in African American patients with sarcoidosis

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To the editor:

Sarcoidosis is a systemic disease that causes the accumulation of granulomas in different organs, most often the lung. Thus, the main cause of mortality is pulmonary fibrosis and respiratory failure in the US (1). Pulmonary fibrosis occurs in twenty percent of sarcoidosis patients and contributes significantly to morbidity and mortality among these patients (2). African Americans have a higher risk of mortality than other racial groups for sarcoidosis (1).

To date, there has been evidence supporting the association of pulmonary sarcoidosis with common genetic variants. Particularly, single nucleotide polymorphisms (SNPs) in certain genes, including *ANXA11* (encoding annexin A11) have been associated with the risk of sarcoidosis patients (3). First discovered in 1977, annexins are a group of calcium regulated membrane bound proteins that have crucial roles in the cell life cycle (4). ANXA11 contains 504 amino acids and has a molecular weight of 56 kDa (5). Three isoforms of ANXA11 are identified in humans, but only one is expressed in human cells, with high expression levels of annexins found in the lung (6). ANXA11is proposed as an anti-apoptotic protein. It is found that genetic variation of ANXA11 increases susceptibility to sarcoidosis based on a previous genome-wide association study (7). Increased activation of $CD4^+$ cells and decreased activation of $CD8^+$ and $CD19^+$, as the most important immune cells in sarcoidosis, has been the proposed mechanism.(7)

However, the role of ANXA11 in pulmonary fibrosis in sarcoidosis has remained unclear. The purpose of this study was to evaluate the association of ANXA11 gene with fibrosing pulmonary sarcoidosis and determine its gene expression and protein levels in blood.

In total 360 consecutive adult subjects diagnosed with sarcoidosis according to the European Respiratory Society (ERS), American Thoracic Society (ATS) and World Association of Sarcoidosis and other Granulomatous Disorders (WASOG) criteria (8) who were seen in the University of Illinois at Chicago (UIC) pulmonary and sarcoidosis clinics were consented to participate in the study

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between January 2010 and January 2015. Among them, 30 African Americans (AA) with pulmonary fibrosis and 36 AA subjects without pulmonary fibrosis (Scadding stage 4 vs. stage 0-1, reviewed by a radiologist expert in thoracic disease) were randomly selected. The mean (SD) of FVC% was 82.6 (20) and 100.2 (20.6) in case and control groups respectively (P=0.004). The Institutional Review Board of the University of Illinois at Chicago approved the study and waived the need for patient consent (approval number of 20130195001).

Targeted genotyping was carried out using the Sequenom iPLEX Gold platform (Reference http://bioscience.sequenom.com/iplex-genotyping). In summary, DNA concentrations were estimated at 10-30 ng/ul using NanoDrop. 2 ul of each DNA sample was PCR amplified and treated with SAP (shrimp alkaline phosphatase). Then, a single base extension was carried out. Reaction products were transferred to SpectroCHIP arrays using the RS1000 Nanodispenser. Spectral analysis was completed using MALDI-TOF mass spectrometer and MassARRAY Analyzer software. Genotyping calls were made by TyperAnalyzer software. Data was curated and reports generated in TyperAnalyzer.

ANXA11 gene expression on peripheral blood mononuclear cells was performed on 15 AA with pulmonary fibrosing sarcoidosis and 15 AA subjects with pulmonary sarcoidosis with stage 0-1. RNA samples were labeled and hybridized according to standard 3' IVT target labeling protocol recommended by Affymetrix. Data was processed using Genomics Suite 6.6 statistical package (Partek, Inc).

Among study cohort, 16 AA subjects with pulmonary fibrosis defined with Scadding score 4 and 25 AA subjects without fibrosis (Scadding stage 0 or 1) were randomly selected to detect *ANXA11* concentration in serum. Mann Whitney test was used to compare mean of levels.

Two SNPs of *ANXA11* (allele T for rs1049550 and C for rs12779955) were found to be significantly associated with pulmonary fibrosis. These differences remained statistically significant after Bonferroni corrections. Allele T frequency for rs1049550 was found to be 4.5 times higher in patients with fibrosis. Allele C frequency for rs127799558 was found to be 8 times higher in fibrotic subjects (Table 1). There was no significant difference in ANXA11 gene expression in PBMC between two groups (P-value=0.97) (Figure 1). Mean (SD) serum ANXA11 levels were 0.97 (0.6841) with minimum of 0.2646 and maximum of 2.613 ng/dl among 36 sarcoidosis subjects. The mean level of serum ANXA11 levels was 0.6441 ng/dl in the case group and 0.6743 ng/dl in the controls. The mean (SD) levels of ANXA11 protein in case and control groups were 0.98 (0.73) ng/dL and 0.90 (0.58) ng/dL respectively. There was no significant difference in circulatory serum ANXA11 protein between two groups (P-value=0.96).

We present the first study showing a relationship between *Annexin A11* SNP (rs1049550) and susceptibility to pulmonary fibrosis amongst patients with sarcoidosis. Patients with African ancestry who carry genotype CT of rs1049550 have 4.5 times higher risk of pulmonary fibrosis. We also found a novel association between *ANXA11* polymorphism (rs12779955) and susceptibility to pulmonary fibrosis in sarcoidosis patients. However, *ANXA11* gene expression and serum protein levels are not altered between two groups without genotype consideration.

Although the mechanistic effect of this change has not been well defined, it appears to affect apoptosis and proliferation in sarcoidosis (7). The mechanism of this increasing resistance to apoptosis was not discussed previously. It is our assumption that *ANXA11* with above SNPs loses all or part of its

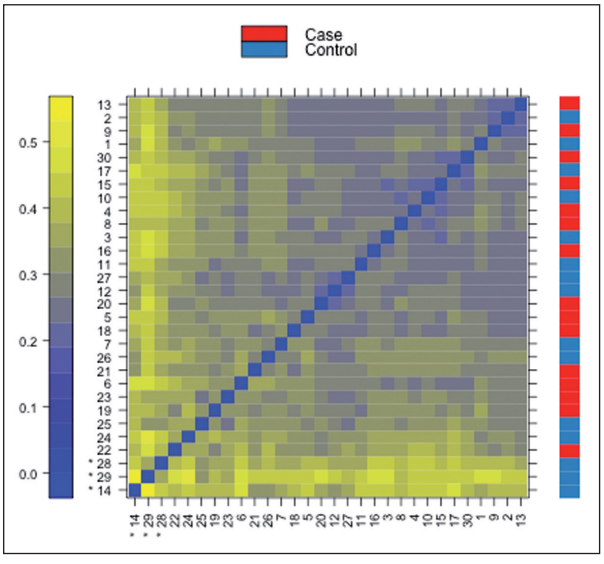

Fig. 1. Showing a false color heatmap of the distances between arrays. The color scale is chosen to cover the range of distances encountered in the dataset. Patterns in this plot can indicate clustering of the arrays either because of intended biological or unintended experimental factors (batch effects)

Table 1. *ANXA11* SNPs frequency in pulmonary sarcoidosis with fibrosis (stage 4) vs. pulmonary sarcoidosis without fibrosis (stage 0-1)

SNPs	African Americans				
	Allele	Case	Control	P value	OR
rs11542745	A	66	72	NS	-
Genotypes					
AA		33	36		
rs115427547	T	1	0	NS	-
Genotypes					
GG		32	36		
GT		1	0		
rs1802932	A	66	72	NS	-
Genotypes					
AA		33	36		
rs1802934	C	66	72	NS	-
Genotypes					
CC		33	36		
rs1802935	C	66	72	NS	-
Genotypes					
CC		33	36		
rs1879201	G	29	34	0.699	-
Genotypes					
GG		6	8		
GA		17	18		
AA		10	10		
rs2228427	A	2	0	0.1368	-
Genotypes					
GG		31	36		
GA		2	0		
rs2229555					
Genotypes	C	66	72	NS	-
CC		33	36		
Rs278986	T	14	20	0.3713	-
Genotypes					
CC		19	17		
CT		14	16		
TT		0	2		
rs3190233	G	1	0	NS	-
Genotypes					
GG		32	36		
GT		1	0		
rs34074920	G	8	5	0.2984	-
Genotypes					
AA		25	32		
AG		6	3		
GG		1	1		
rs24414015	A	0	2	NS	-
Genotypes					
GG		33	35		
AG		0	1		

(continued)

Table 1 *(continued)*. *ANXA11* SNPs frequency in pulmonary sarcoidosis with fibrosis (stage 4) vs. pulmonary sarcoidosis without fibrosis (stage 0-1)

SNPs	African Americans				
	Allele	Case	Control	P value	OR
rs35715926	A	0	2	NS	-
*Genotypes**					
GG		33	34		
GA		0	1		
rs4130868	C	66	72	NS	-
Genotypes					
CC		33	36		
rs61860018	G	66	72	NS	-
Genotypes					
GG		33	36		
rs61862361	C	66	72	NS	
Genotypes					
CC		33	36		
rs6585454	G	10	5	0.1152	
Genotypes					
AA		24	31		
GA		8	5		
GG		1	0		
rs12779955	C	7	1	0.0206	8.4 (1.01-70)
Genotypes					
TT		27	35		
CT		5	1		
CC		1	0		
rs1049550**	T	9	7	0.016	4.5 (1.3-15.9)
Genotypes					
CC		8	28		
CT		9	7		

* rs35715926 was studied in 33 cases and 35 controls; ** rs1049550 was studied in 17 cases and 35 controls

functionality. ANXA11 carries 4 calcium ions and delivers calcium to many intracellular pathways. ANXA11 is involved in apoptosis in at least two known pathways. It is involved in mitogen-activated protein kinase (MAPK) and P53 pathways. Mitogen-activated protein kinase pathways are involved in apoptosis in the setting of environmental stress (9). The MAPK pathway activates caspase pathway via an ALG-2 protein that is Ca^{2+} dependent. Without calcium delivery from ANXA11 to ALG-2, the apoptosis via caspase pathway would not be activated (10, 11).

The current preliminary study has several limitations. We do not have a validation cohort to confirm our findings. No assessment of lung levels of the *ANXA11* gene and protein expression was performed.

If the potential role of *ANXA11* SNPs in increasing susceptibility to fibrosing sarcoiodosis is validated, a new opportunity to develop a prognostic test in sarcoidosis will be recognized.

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Conception, review literature, design and modeling for review writing manuscript: M.M., R.F.M The review literature, design and modeling for review writing manuscript: M.M., R.F.M, C.Z,T.A, A.H, D.S, N.S,Z.A. Writing the article or substantial involvement in its revision before submission: M.M, M.F.M., J.G, D.S. R.B

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