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Genome-Wide Association Study of 58 Individuals with Fibrosing Mediastinitis Reveals Possible Underlying Genetic Susceptibility

To the Editor:

Histoplasma capsulatum is a dimorphic fungus that is endemic to the Mississippi and Ohio river valleys in the United States and isolated pockets throughout the world (1). Although most adults in endemic areas have evidence of prior infection based on skin testing to histoplasmin (1, 2), infection is asymptomatic or produces mild respiratory symptoms only in most patients with an intact immune system (3).

A late and uncommon complication of prior infection with *H. capsulatum* is fibrosing mediastinitis (FM). In FM, fibrotic and often calcified tissue invades the mediastinum and may obstruct or occlude central vessels and airways (4). Unilateral disease is usually well tolerated in otherwise healthy individuals, but in our experience, bilateral disease is often fatal. Evidence linking FM to prior histoplasmosis includes occurrence primarily in endemic areas, prior clinical diagnosis of histoplasmosis in some patients, and the direct visualization of organisms within mediastinal fibrosis (4). Similar mediastinal lesions can also result from idiopathic pulmonary fibrosing mediastinitis, which is usually accompanied by extrathoracic fibrosis and has been linked to IgG4-related disease (5). At our center, idiopathic pulmonary fibrosing mediastinitis represents a small minority of patients who present with clinical signs of FM.

Despite near-ubiquitous infection with *H. capsulatum* in endemic areas, FM appears to be uncommon. We hypothesized that this discrepancy relates to underlying genetic susceptibility in affected individuals. Therefore, we performed a genome-wide association study in 58 individuals with FM recruited between 2010 and 2016 and compared it with 7,115 nondiseased controls from a DNA biobank (BioVU cohort), as well as controls from the 1000 Genomes Project (6).

The definition of fibrosing mediastinitis was established by one of the study investigators (J.E.L.) based on compatible clinical and radiologic features (7). Each patient with FM provided written informed consent under a protocol approved by the Institutional

Review Board of Vanderbilt University Medical Center. Control DNA samples (BioVU cohort) were randomly selected from an existing DNA biobank populated by samples obtained from unused and otherwise discarded blood originally obtained for clinical purposes (8). Samples were selected in an 8:1 ratio of white non-Hispanic to black patients based on the race distribution of the cases, and were filtered to remove FM cases. Genotyping of genomic DNA was performed on an Expanded Multi-Ethnic Genotyping Array (MEGA^{EX}) Vanderbilt Custom DNA BeadChip (Illumina) platform, which contains 2,036,060 total SNPs. After hybridization, staining, and washing steps, beads were scanned and the resulting scan data were loaded into Genome Studio v2010.2 for analysis. The mean call rate for experimental samples and controls/duplicates was 98.02%. Reproducibility and heritability frequencies for directly genotyped alleles were all above 99.9% for two duplicates and one HapMap trio, indicating excellent concordance and Mendelian heritability. Some of the results of these studies have been previously reported in the form of an abstract (9).

Patients with FM were predominantly white non-Hispanic and had unilateral vessel involvement (Table 1). Using an α threshold of $P < 2.5 \times 10^{-8}$ and a minor allele frequency cutoff of 5% in the FM cases, we identified 270 exonic SNPs that differed significantly between FM cases and BioVU controls. A P value of 2.5×10^{-8} was selected based on Bonferroni correction ($0.05/2,036,060$ individual SNPs). To further examine these findings, we then compared the minor allele frequency of each of these SNPs in patients with FM with an independent control population consisting of subjects from the 1000 Genomes project (10). Corresponding sequencing data in 1000 Genomes was available for 261 (97%) of the SNPs identified in the BioVU cohort. For 254 of the 261 SNPs, the direction of association was opposite that of the discovery analysis, or the association did not reach statistical significance ($P < 0.05$). Gene ontology analysis showed that the remaining 7 SNPs did not cluster along a distinct molecular function, biological process, or signaling pathway, and that each was present in a small minority of patients with FM (Table 2). An additional nine SNPs that met our threshold for significance in the BioVU cohort but did not have corresponding sequencing data available in 1000 Genomes are also listed in Table 2. Interestingly, the *HYDIN* (hydrocephalus-inducing protein homolog) gene was represented by three unique SNPs in this group.

The specific cell type or types that contribute to the development of the FM phenotype are unknown. We questioned, however, whether the SNPs that were differentially expressed in patients with FM were expressed in the human lung or by the lung immune cells. Using the

Table 1. Clinical Characteristics of 58 Patients with Fibrosing Mediastinitis

Age, yr, mean (range)	40 (19–71)
Male sex, <i>n</i> (%)	28 (48)
Race, <i>n</i> (%)	
White non-Hispanic	49 (84)
Black	9 (16)
Vessel involvement	
Unilateral, <i>n</i> (%)	44 (76)
Bilateral, <i>n</i> (%)	14 (24)
Airway involvement	
Mainstem bronchus, <i>n</i> (%)	2 (3)

This work was supported by National Institute of Child Health and Human Development grant HD043483-16, NHLBI grant HL138088, and National Center for Advancing Translational Sciences grant UL1TR000445. The BioVU dataset was obtained from Vanderbilt University Medical Center's BioVU, which is supported by institutional funding, the 1S10RR025141-01 instrumentation award, and the Clinical and Translational Science Awards grant UL1TR000445 from the National Center for Advancing Translational Sciences.

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Table 2. Differentially Expressed SNPs between Patients with Fibrosing Mediastinitis and Control Subjects

SNP	Gene	A1	A2	MAF Cases	MAF Controls (BioVU)	MAF Controls (1000 Genomes)	P (BioVU)	P (1000 Genomes)
rs143105143 (missense)	MRPL44	T	G	7.27%	0%	0.04%	9.35×10^{-18}	1.23×10^{-10}
7:45122058 (missense)	NACAD	T	G	9%	0.09%	0.13%	9.29×10^{-15}	5.06×10^{-11}
rs7728764 (synonymous)	NKX2-5	T	C	5.56%	0%	0.69%	1.6×10^{-13}	2.87×10^{-4}
rs146674681 (missense)	IRX4	A	G	5.56%	0%	0.22%	1.61×10^{-13}	2.66×10^{-4}
rs57550068 (missense)	NOC3L	T	C	5.08%	0.01%	1.77%	7.53×10^{-12}	2.43×10^{-2}
rs35381228 (splice region)	ABL1	T	C	5.08%	0.03%	1.95%	5.57×10^{-11}	3.49×10^{-2}
rs78841685 (missense)	CD97	T	C	5.08%	0.03%	1.04%	5.61×10^{-11}	2.63×10^{-3}
8:143781950 (missense)	LY6K	C	T	23.64%	0.28%	—	5.82×10^{-39}	—
4:77069485 (synonymous)	NUP54	A	C	10.91%	0%	—	2.25×10^{-26}	—
11:118917318 (missense)	HYOU1	T	C	10.19%	0%	—	2.64×10^{-24}	—
16:70897062 (synonymous)	HYDIN	T	C	11.21%	0.37%	—	3.68×10^{-15}	—
16:70942706 (synonymous)	HYDIN	T	A	6.78%	0.18%	—	1.93×10^{-10}	—
rs34292278 (missense)	SMTN	T	C	6.78%	0.20%	—	4.12×10^{-10}	—
19:44377930 (synonymous)	ZNF404	T	G	7.76%	0.32%	—	5.03×10^{-10}	—
16:70917997 (missense)	HYDIN	A	C	5.08%	0.10%	—	9.61×10^{-9}	—
rs74128855 (missense)	SYT15	A	G	5.08%	0.12%	—	2.46×10^{-8}	—

Definition of abbreviation: MAF = minor allele frequency.

For the BioVU cohort, see Reference 8. For the 1000 Genomes Project, see Reference 10.

Lung Gene Expression Analysis web portal (11), we found that all the genes in Table 2 except *CD97* were expressed in the human lung, but only *MRPL44* (mitochondrial ribosomal protein L44), *NOC3L* (NOC3-like DNA replication regulator), and *ABL1* (ABL proto-oncogene 1, nonreceptor tyrosine kinase) were expressed by lung immune cells.

The small sample size of our case cohort and the lack of a replication cohort created significant limitations for this study and reflect the rarity of this disease. These data should be viewed as exploratory, pending replication in an independent cohort. However, our results suggest that FM may have a genetic basis and open the door for future investigations into the genetic underpinnings of this enigmatic disease. ■

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Accuracy of Models to Identify Lung Nodule Cancer Risk in the National Lung Screening Trial

To the Editor:

Clinical models to estimate the probability of cancer in pulmonary nodules detected incidentally on imaging tests are used both in the clinic setting and for research purposes (1). Recently, the Brock model was developed for nodules detected by low-dose computed tomography screening (2). Patients with incidentally detected nodules and those with screening-detected nodules have different risk profiles because those screened for lung cancer must have a number of risk factors for lung cancer, whereas the patients with incidentally detected nodules do not.

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