

Online Data Supplement

Enlarged Dural Sac in Idiopathic Bronchiectasis Implicates Heritable Connective Tissue Gene Variants

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Methods:

The local institutional review board approved the present study. Informed consent and MRI safety screening forms were completed in prospectively studied subjects, but informed consent was waived for evaluation of pre-existing MR and CT images. All data were recorded in a Health Insurance Portability and Accountability Act (HIPAA)-compliant protected database.

Study Subjects

Measurements were obtained from four groups of adults (≥ 18 years of age). Idiopathic bronchiectasis subjects (n=71 subjects from 66 families) included individuals with bronchiectasis in two or more lobes, <10 pack year smoking history, and exclusion of other potential etiologies including cystic fibrosis, primary ciliary dyskinesia, alpha-1 antitrypsin deficiency, ABPA, inflammatory bowel disease, autoimmune disease, immune deficiency, prior solid organ transplant, and prior history of tuberculosis. None of the IB subjects met the clinical criteria required for a diagnosis of HCTD. Control subjects (n=72) without lung or spinal disease or evidence of HCTD were imaged as previously described (1). Cystic fibrosis (CF) subjects (n=29) were confirmed to carry two disease-causing mutations in *CFTR* and served to control for the possibility that coughing might cause increased intracranial and intrathecal pressure and affect dural size. An additional 24 subjects carried a genetic or clinical diagnosis of MFS and had a pre-existing MRI or CT examination of the lumbo-sacral spine available for measurement (1). Exclusion criteria for all subjects included severe thoracic or lumbar scoliosis (Cobb angle of 30 degrees or more), thoracic or lumbar spine surgery, spinal stenosis, or spine injury. All idiopathic bronchiectasis subjects had prospective imaging performed. Because it would pose a significant burden to recruit and enroll Marfan subjects, we used pre-existing imaging (CT or

MR). 53 control and 22 cystic fibrosis patients had pre-existing MRIs ordered for clinical reasons, such as "pain" or "trauma". If the pre-existing study was read by the attending radiologist as abnormal or if the study had spine pathology that might potentially alter the vertebral column or dural sac, it was not included (exclusion criteria for all subjects included severe thoracic or lumbar scoliosis (Cobb angle of 30 degrees or more), thoracic or lumbar spine surgery, spine injury, and acquired spinal stenosis). We prospectively imaged additional subjects in both of these groups (19 control and 7 cystic fibrosis) to increase our statistical power. Because we found no difference in dural sac diameter between control subjects with pre-existing imaging and control subjects with prospective imaging (Daniels et al. (1), we felt that use of pre-existing imaging and prospective imaging resulted in minimal selection bias.

Imaging Protocol and Analysis

Both pre-existing and prospective lumbar MRI examinations were performed as described in Daniels et al. (1) All exams were reviewed by a board-certified radiologist with >10 years of experience (Reader 1) and a second-year medical student (Reader 2), both of whom were blinded to diagnosis. Reader 2 was trained on measurement techniques by the radiologist, and completed several practice cases under supervision before measuring study cases. Mid-sagittal images were used to measure the anterior-posterior dural sac diameter (AP-DSD) from L1 to L5. Axial or axial-oblique images were used to measure the orthogonal transverse (TR) DSD. If needed, images were manipulated with the 3D PACS tool in order to obtain true orthogonal measurements. Dural sac measurements were made at the mid-corpus level of the vertebral body, perpendicular to the long axis of the dural sac.

Determination of Phenotypic Features

Pectus abnormalities were classified as pectus excavatum or pectus carinatum using a corrected Haller Index (2) or if there was a sternal tilt resulting in chest wall asymmetry that was noted on physical exam or chest imaging. Scoliosis was defined as presence of a single spinal curve with a Cobb angle of 8 degrees or more or the presence of two spinal curves, each having a Cobb angle of at least 5 degrees as measured from a posterior-anterior chest radiograph using the Cobb method (3).

Mitral valve prolapse was defined by findings of redundant mitral valve leaflets with prolapse into the left ventricle noted on an echocardiogram, but presence of mitral regurgitation was not required. Fingers that are disproportionately long as compared to the palm of the hand were assessed by the (Walker) wrist sign and the (Steinberg) thumb sign. A positive wrist sign is present when the thumb tip covers the entire fingernail of the fifth finger when wrapped around the opposite wrist. A positive thumb sign is present when the entire distal phalanx of the adducted thumb projects beyond the ulnar border of the palm (4, 5). A high-arched palate is present when the maximum palate height is greater than twice the height of the teeth (6). Joint hypermobility was determined by the Brighton criteria (7). This includes the Brighton score (7), which requires quantified assessment of hyperextension using a goniometer. If joint hypermobility could not be assessed in person, a validated 5-point questionnaire for generalized joint hypermobility (8) was fulfilled by phone or email.

Methods to Determine Clinical Features

Lung function was measured using pre-bronchodilator spirometry measurements obtained according to ATS guidelines (9). Percent predicted values were calculated using published

reference values (10). The presence of bronchiectasis was determined for each lobe of the lung; the lingula was considered as the 6th lobe (separate from the left upper lobe). Severity of bronchiectasis was classified as mild, moderate, or severe as defined in **Table E1** in the online data supplement.

For infection status, individuals with at least one expectorated sputum sample or bronchoscopically obtained respiratory sample sent for bacterial culture were classified according to the presence or absence of *Pseudomonas aeruginosa*. Individuals with at least two expectorated sputum sample or one bronchoscopically obtained respiratory sample sent for NTM culture were classified according to the presence or absence of pathogenic forms of non-tuberculous mycobacterium, such as *M. avium*, *M. abscessus*, and *M. kansasii*. All of these individuals also had radiographic findings consistent with pulmonary NTM infection based on the ATS/IDSA guidelines (11).

Individuals were considered to have a family history of bronchiectasis if at least one first or second degree relative had been diagnosed with bronchiectasis, and no etiology of the bronchiectasis could be determined after a medical evaluation. When possible, objective data was used to confirm the diagnosis of bronchiectasis.

Statistical Analysis

Linear regression was performed to compare the effects of covariates, including height, gender, and race on AP-DSD and TR-DSD measurements of L1 through L5 and the L1-L5 (average). For the effect of the covariate of age, we regressed on the oldest patient per family (n = 66).

Pearson's correlation was used to calculate the unadjusted correlation between readers 1 and 2 for the AP-DSD and TR-DSD measurements. Measurements for AP-DSD and TR-DSD by reader 1

were compared between all four groups using pairwise comparison of means. Tukey method was used to adjust for multiple comparisons. Within the idiopathic bronchiectasis group, comparison of L1-L5 (average) AP-DSD by individual dichotomous clinical characteristic variables was performed using a student t-test. For comparison of males and females within the IB group, student t-test was used for continuous variables including FEV1, BMI, age, height. Chi-square and Fisher exact test were used for comparison of categorical variables such as Pseudomonas and NTM positivity, radiographic findings consistent with NTM disease, and family member with bronchiectasis. Wilcoxon-Mann-Whitney was used for comparison of lobes with bronchiectasis. For all tests, $p < 0.05$ indicated a statistically significant difference. All statistical analysis was performed using STATA (StataCorp. 2013. *Stata Statistical Software: Release 13*. College Station, TX: StataCorp LP.)

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Tables:

Table E1: Bronchiectasis Severity Scoring Method for Each of Six Lobes

	Score	Bronchi/bronchiole dilation with respect to adjacent artery	Related distortion of bronchovascular bundle or lung architecture	Endobronchial or endobronchiole mucus plugging	Cicatrization or Collapse
Absent	0	None	Absent	Absent	Absent
Mild	1	Up to 25%	Absent	May be present	Absent
Moderate	2	25-50%	May be present	May be present	Absent
Severe	3	> 50%	Present	Present	May be present
Surgically Resected	4	N/A	N/A	N/A	N/A

Definition of abbreviations: N/A = not available

Table E2: Distribution of Bronchiectasis

	None	Mild	Moderate/Severe/Resected
LUL; # (%)	24 (34%)	42 (60%)	5 (7%)
RUL; # (%)	15 (21%)	46 (65%)	10 (14%)
Lingula; # (%)	14 (20%)	26 (37%)	31 (44%)
RML; # (%)	8 (11%)	24 (34%)	39 (55%)
LLL; # (%)	11 (16%)	38 (54%)	21 (30%)
RLL; # (%)	16 (23%)	32 (46%)	22 (31%)

Definition of abbreviations: LUL = left upper lobe; RUL = right upper lobe; RML = right middle

lobe; LLL = left lower lobe; RLL = right lower lobe

Table E3: Prevalence of Phenotypic Features Among Idiopathic Bronchiectasis Group

	IB Female (N = 53)	IB Male (N = 18)	IB Total (N = 71)	Population	Control Group
Scoliosis	21% (11/53)	22% (4/18)	21% (15/71)	0.5-3.2% †(12)	2.2% (1/45)
Pectus *	47% (25/53)	11% (2/18)	38% (27/71)	<1% †(12)	2.4% (1/42)
MVP	57% (13/23)	57% (4/7)	57% (17/30)	2.4-2.7% §(13)	N/A
Brighton	35% (16/46)	50% (7/14)	38% (23/60)	5-17% ¶(14)	N/A
Wrist Sign †	32% (16/50)	12% (2/17)	27% (18/67)	7.7% **(5)	N/A
Thumb Sign	4% (2/50)	12% (2/17)	6% (4/67)	4.7% **(5)	N/A

High Arched Palate	27% (13/49)	35% (6/17)	29% (19/66)	15.2% ** (5)	N/A
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Definition of abbreviations: IB = idiopathic bronchiectasis; MVP = mitral valve prolapse; N/A =

not available

Values are % (number positive/number with available data)

* p=0.005 (IB female vs IB male)

† p=0.091 (IB female vs IB male)

‡ Reported in Leung et al. 2013

§ Reported in Freed et al. 1999

|| Reported in Fikree et al. 2013

** Reported in Sponseller et al. 2010

Table E4: Group Demographics:

	Control (N = 72) *(1)	IB (N = 71)	Marfan (N = 24) *(1)	CF (N = 29)
Female; N (%)	35 (49%)	53 (75%)	10 (42%)	17 (59%)
Age, years; mean (range)	47.7 (18 - 88)	57.3 (18 - 89)	36.9 (20 - 74)	34.3 (19 - 60)
BMI; mean (range)	27.9 (16.7 - 44.0)	23.6 (12.9 - 47.8) †	24.6 (18.1 - 32.4)	21.3 (15.0 - 31.6) †
Height, cm; mean (range)				
Male	176.7 (160.0 - 188.0)	177.0 (156.6 - 187.6)	191.8 (175.3 - 208.3) §	173.4 (162.02-184.0)
Female	162.7 (151.1 - 177.8)	162.7 (149.5 - 175.5) ‡	179.7 (164.1 - 190.5) §	158.3 (150.0 - 173.0)
Race/Ethnicity; N (%)				
White	56 (78%)	63 (89%)	12 (50%)	27 (93%)

African American	10 (14%)	3 (4%)	10 (42%)	1 (3.5%)
Other or Unknown	2 (3%)	3 (4%)	1 (4%)	0 (0%)

Definition of abbreviations: IB = idiopathic bronchiectasis; CF = cystic fibrosis; BMI = body mass index

* Reported in Daniels et al. 2015.

† p < 0.001 as compared to control group (F 23.2; M 24.9)

‡ p = 0.05 as compared to CF group

§ p < 0.001 as compared to control group

Table E5: Pearson Correlation Coefficient Between Readers

	L1 AP-DSD	L2 AP-DSD	L3 AP-DSD	L4 AP-DSD	L5 AP-DSD	L1-L5 (average) AP-DSD
Control (N = 72)	0.77	0.82	0.75	0.86	0.85	0.90
IB (N = 71)	0.75	0.82	0.86	0.86	0.88	0.93
Marfan (N = 24)	0.68	0.80	0.80	0.77	0.71	0.87
CF (N = 29)	0.72	0.78	0.69	0.89	0.86	0.87

	L1 TR-DSD	L2 TR-DSD	L3 TR-DSD	L4 TR-DSD	L5 TR-DSD	L1-L5 (average) TR-DSD
Control (N = 72)	0.72	0.74	0.76	0.79	0.78	0.87
IB (N = 71)	0.64	0.71	0.71	0.76	0.85	0.90
Marfan (N = 24)	0.81	0.82	0.77	0.87	0.85	0.90
CF (N = 29)	0.76	0.71	0.81	0.79	0.82	0.87

Definition of abbreviations: IB = idiopathic bronchiectasis; CF = cystic fibrosis; AP-DSD = anterior-posterior dural sac diameter; TR-DSD = transverse dural sac diameter

Table E6: AP-DSD Measures (Reader 1)

	L1	L2	L3	L4	L5	L1-L5 (average)
Control (N = 72)	1.51 ± 0.16	1.42 ± 0.15	1.35 ± 0.16	1.34 ± 0.19	1.28 ± 0.20	1.38 ± 0.15
IB (N = 71)	1.66 ± 0.15	1.58 ± 0.19	1.50 ± 0.19	1.46 ± 0.21	1.41 ± 0.26	1.52 ± 0.18 *
Marfan (N = 24)	1.62 ± 0.17	1.59 ± 0.19	1.59 ± 0.18	1.60 ± 0.23	1.67 ± 0.30	1.61 ± 0.17 †
CF (N = 29)	1.51 ± 0.16	1.47 ± 0.17	1.39 ± 0.17	1.32 ± 0.18	1.27 ± 0.21	1.39 ± 0.15 †

Definition of abbreviations: IB = idiopathic bronchiectasis; CF = cystic fibrosis; AP-DSD = anterior-posterior dural sac diameter.

Values are mean ± SD

† IB is significantly different from controls ($p < 0.001$)* and from CF ($p=0.002$).

‡ Marfan is trending towards a significant difference as compared to IB ($p = 0.084$).