SUPPLEMENTAL MATERIALS

Supplemental Acknowledgements

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Supplemental Methods

Cell culture cytokine analysis

Peripheral blood mononuclear cells (PBMC) from previously genotyped wildtype *CLEC7A* subjects (AA) and *CLEC7A* heterozygotes (AC) at an initial concentration of 5 $x10^6$ were thawed from -80ºC and rested overnight. Cells ($1x10^5$) were incubated for 4 hours in R10 media (RPMI supplemented with 10% human serum) with the dectin-1 agonist zymosan (10 ug/mL, Sigma-Aldrich, #58856-93-2) and tacrolimus (10 ng/ml, VWR International), or with 150 ul of R10 media as a negative control. Cytokines were measured from supernatant using multiplex ELISA (Discovery Assay, Eve Technologies).

Supplemental Results

Cytokine production is intact in *CLEC7A* variants following zymosan stimulation

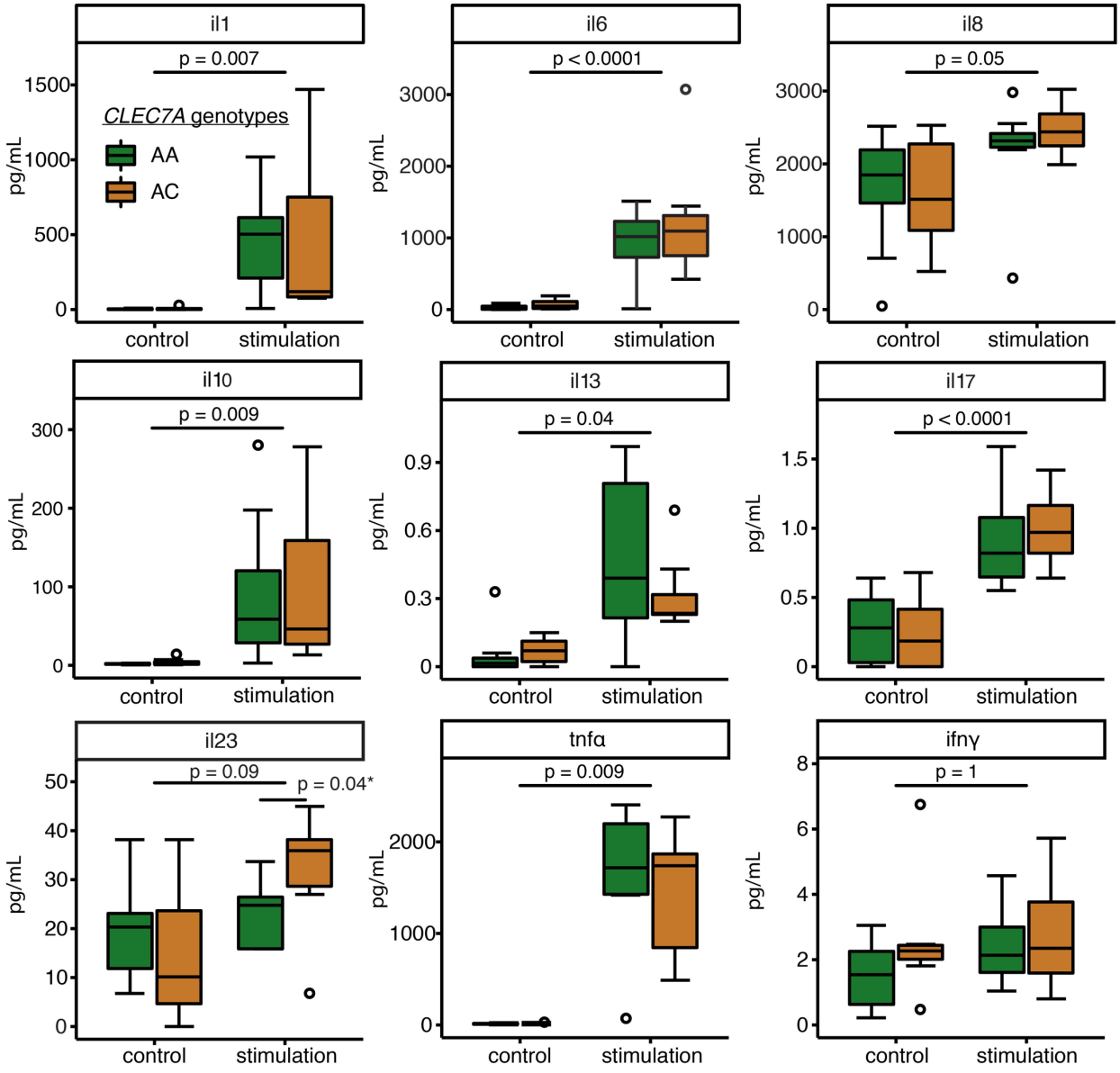
Cytokines important for CD4 T-cell signaling were measured from cell culture supernatant after stimulation with the dectin-1 agonist zymosan. Because zymosan also signals via TLR-2 in an NFAT-dependent manner, which may be inhibited by tacrolimus immunosuppression, we also included tacrolimus in the stimulation assays. The results of experiments from 8 subjects heterozygous for *CLEC7A* AC loss-of-function polymorphisms and 8 subjects with wildtype *CLEC7A* genotypes are displayed in Supplemental Figure 1. Several cytokines in supernatant were increased after 4 hours of stimulation compared to control: IL-1, IL-6, IL-8, IL-10, IL-13, IL-17, IL-23, and TNFα. Notably, IFN-gamma, IL-2 and IL-12 concentrations were too low to detect reliably in this assay. No statistically significant differences in cytokine production was observed between genotypes after adjustment for multiple comparisons.

Y238X carriers were more likely to experience an increase in chronic immunosuppression after bronchoscopy.

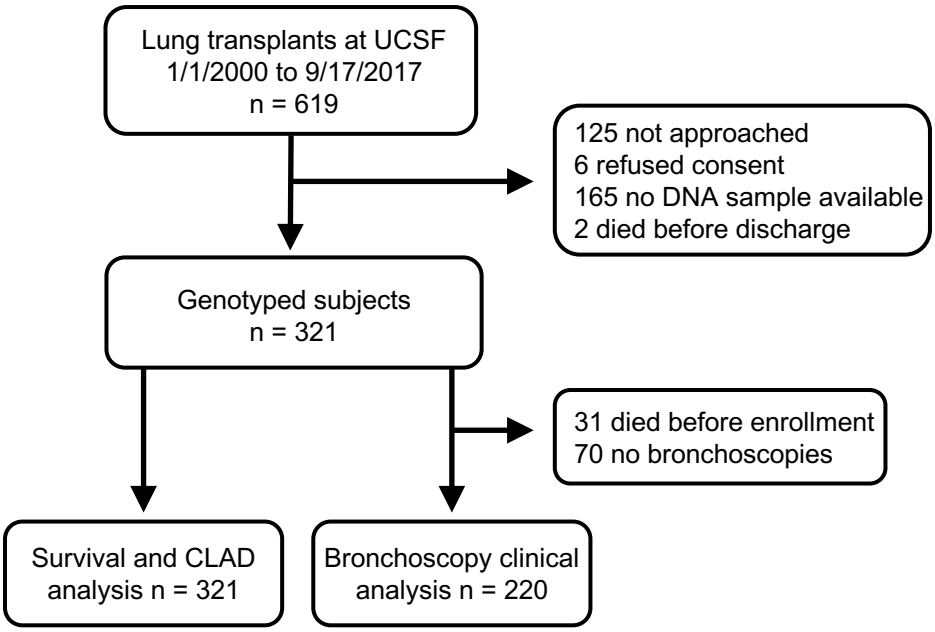
This database contained 1134 bronchoscopy encounters for 220 subjects with a median of 16 bronchoscopies documented per subject. Bronchoscopies were most often performed for surveillance purposes with cough being the most reported sign or symptom at the time of bronchoscopy. We had 246 cases of acute cellular rejection with 61% occurring within the first year following transplantation. The most common type of pathogen identified was bacteria; though, the most common organism was Rhinovirus (n = 99). The most common fungus identified was Aspergillus. Details of the classes of microbes identified during the study period can be found in Supplemental Table 3. There were no differences in the frequencies of bacteria, fungus, virus, or Acid Fast Bacillus isolated by genotype. Supplemental Figure 3 contains the results from the generalized estimating equation analysis comparing bronchoscopy endpoints between Y238X

carriers and subjects without dectin-1 polymorphisms. We found no statistically significant differences between bronchoscopy indication, signs and symptoms, or pathology findings between the dectin-1 genotypes. Compared to wildtype subjects, dectin-1 carriers were 3.2 times more likely to experience an increase in chronic immunosuppression following bronchoscopy (CI 1.4-7.6, $p = 0.008$) and 0.6 times less likely to be treated for an acute infection (CI 0.3-0.9, $p = 0.027$).

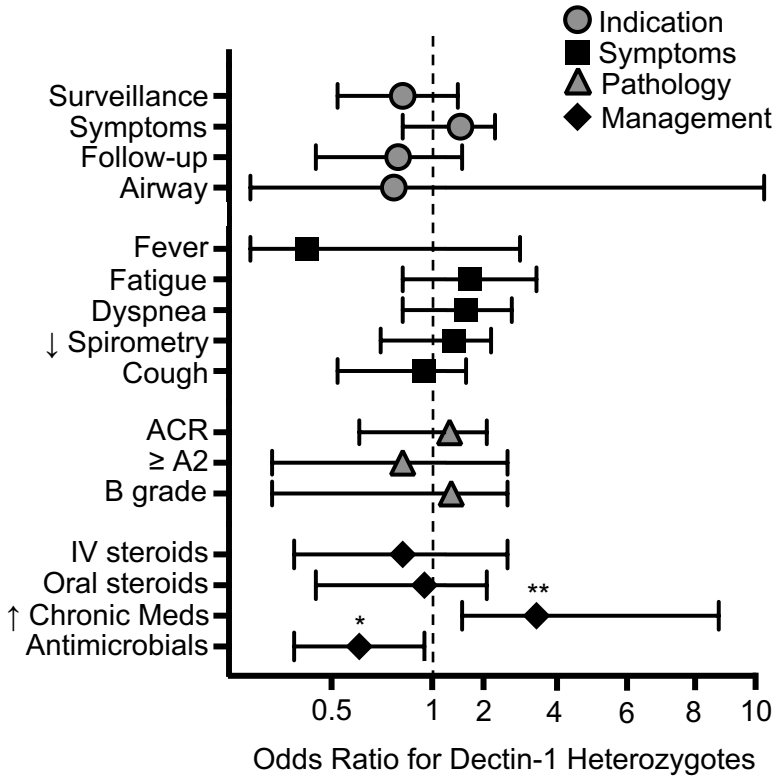
Supplemental Figure 1. Similar cytokine production following stimulation between Y238X variant AC and *CLEC7A* wildtype individuals. Box and whisper plots depict cytokine concentrations of cell culture supernatant after 4 hours of zymosan and tacrolimus stimulation from dectin-1 wildtype (AA, green, n = 8) and heterozygous (AC, orange, n = 8) subjects. Several cytokines in supernatant were increased after 4 hours of stimulation compared to control. There was no difference in the concentration of TNF-alpha after stimulation. Differences were assessed by Student's t-test adjusted for multiple comparisons as described by Benjamini and Hochberg. *In an analysis unadjusted for false discover rate (fdr), IL-23 was increased in the stimulated dectin-1 heterozygous supernatant (36 IQR 29 - 38 pg/mL) compared to stimulated wildtype supernatant (25 IQR 16 - 26 pg/mL, $p = 0.04$ fdr unadjusted, p = 0.1 fdr adjusted).



Supplemental Figure 2: Subject inclusion and exclusion diagram for the UCSF cohort.



Supplemental Figure 3: Associations between dectin-1 genotypes and bronchoscopy procedure indication and outcome. There were no differences in bronchoscopy indication, signs symptoms at bronchoscopy encounter, or pathology findings from transbronchial biopsy. Dectin-1 carriers (AC and CC) demonstrated decreased odds of being treated with antimicrobials (OR 0.6, CI 0.3-0.9, p = 0.027) and increased odds of undergoing an increase in their chronic immunosuppression (OR 3.2, CI 1.4-7.6, *p* = 0.008) compared to wild type subjects. Legend: ↓ Spirometry = changes in forced vital capacity or forced expiratory volume in 1 second, ↑ Chronic Meds = increased chronic immunosuppression.



Supplemental Table 1: Dectin-1 Protein by CLAD Phenotype. Samples from this nested analysis are from the UCSF cohort and were matched on age and time after transplant.

	CLAD	No CLAD	*p* value
Subjects (n)	25	20	
Age at transplantation, mean years ± SD	56 ± 11	56 ± 13	0.73
Male gender (%)	50	56	0.73
Transplant type: N (%)			0.74
Double	22 (88)	18 (90)	
Heart and Lung	0	0	
Single	3 (12)	2 (10)	
Race/Ethnicity: N (%)			0.79
Caucasian	18 (72)	16 (80)	
African American	0	0	
Hispanic	5 (20)	2 (10)	
Other	2 (8)	1 (5)	
Transplant indication group: N (%)			0.73
A (COPD)	6 (24)	4 (20)	
B (Pulmonary Hypertension)	2 (8)	1 (5)	
C (Cystic Fibrosis)	0	0	
D (Pulmonary Fibrosis)	18 (72)	15 (75)	

Supplemental Table 2: Characteristics of subjects included in prospective bronchoscopy analysis and immunophenotyping analysis compared to subjects not included from genotyping cohort.

	Entire Cohort	Bronchoscopy Analysis	*p*
Subjects: N	321	220	
CLEC7A genotypes			0.72
AA	280	192	
AC&CC	41	28	
Age at transplant, mean years ± SD	55 ± 12	55 ± 12	.35
Male gender (%)	54	57	0.07
Transplant type: N			0.81
Double	288	198	
Other	33	22	
Race/Ethnicity: N			0.07
Caucasian	241	158	
Other	80	62	
Indication: N			0.1
D (IPF)	210	156	
A,B,C	111	64	

Supplemental Table 3: Findings of the bronchoscopy culture data showing no differences between genotypes in the frequencies of the 10 most commonly isolated microbes on bronchoscopy. There was also no difference in the frequency of isolation of any bacteria, virus, fungus, or Acid Fast Bacillus. There were 1134 bronchoscopies performed during the study period (AA genotype, n=999; AC and CC genotypes, n = 135).

Microbe	AA genotype	AC or CC genotype	*p* value
Penicillium	187	22	0.6
Rhinovirus	90	9	0.5
Aspergillus species	83	11	1
Haemophilus parainfluenza	68	7	0.6
Pseudomonas aeruginosa	50	5	0.7
Staphylococcus aeruginosa	27	2	0.6
Stenotrophomonas maltophilia	21	6	0.2
Mycobacterium gordonae	12	3	0.6
Escherichia coli	10	0	0.5
Klebsiella species	9	1	1
Any bacteria	975	128	0.1
Any virus	125	15	0.8
Any fungus	341	40	0.3
Any Acid Fast Bacilli	18	4	0.6

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