(i) Exome sequencing to find rare variants influencing lung function decline in COPD

(ii) Exome sequencing to find rare variants determining risk for PAH in SSc individuals

March 28th, 2012

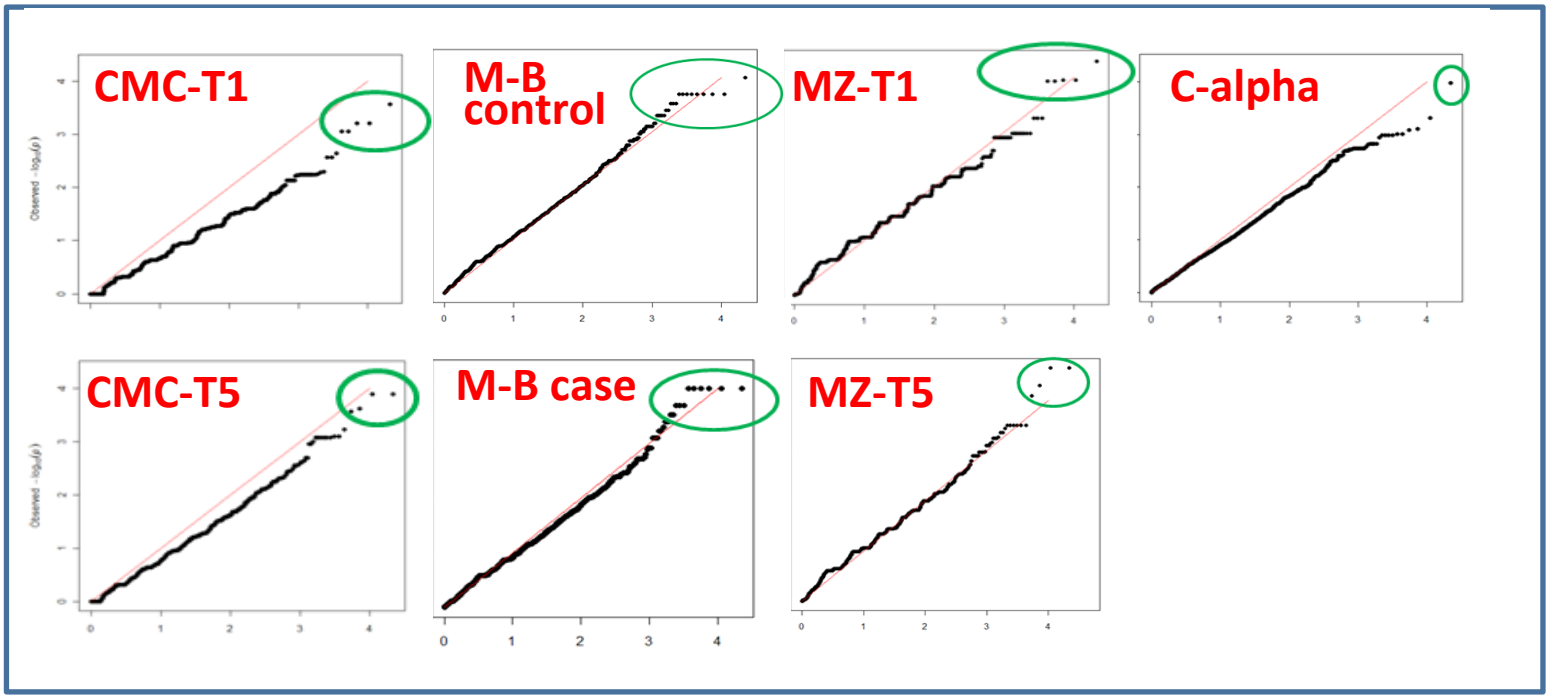
LHS Analysis Pipeline & Results

336 INDIVIDUALS

174 CASES(FAST)/ 162 CONTROLS(SLOW)

VARIANT N= 80,162 (FILTERED AUTOSOMAL NON-SYN)

TRANSCRIPT N=24,142



Gene	CHR	# nonsyn SNPs	# RV MAF<5%	Fast Decliner		Slow Decliner		Min Pvalue (corresponding burden test)	Number of singletons in LHS exomes (*)	Distribution of singletons by fast/slow status
				RV+	RV-	RV+	RV-			
ADAM12	10	8	7	27	147	10	152	0.00005 (MZ-T1)	3 (2)	All 3 fast decliners
DCAF5	14	6	6	0	174	11	151	0.00012 (MZ-T1)	4 (4)	All 4 slow decliners
HK2	2	9	8	16	156	1	157	0.00008 (MZ-T5)	6 (3)	5 = fast decliner 1 = slow decliner
MTRR	5	14	10	23	151	8	154	0.00019 (MB-case)	4 (3)	All 4 fast decliners

RV = rare variants

ADAM12: Modulation of RNA levels of ADAM12 has been demonstrated in induced sputum from asthma patients. ADAM12 has been implicated in the TGFbeta signaling pathway, a pathway with known significance in COPD pathogenesis, and has been shown to be expressed in lung tissue, epithelial and sputum cells.

DCAF5: encodes a cullin-RING ubiquitin ligase that regulates DNA repair, DNA replication and transcription. A recent study demonstrating interactions between nicotine dose, dependence and a 'quit-success' genotype score derived from a recent GWAS implicated DCAF54.

MTRR: variants in MTRR (5-methyltetrahydrofolate-homocysteine methyltransferase reductase) have been associated with hyperhomocysteinemia in the Framingham cohort5, and studies have shown that patients with COPD have poor vitamin B status and consequently, increased plasma total homocysteine (tHcy), placing them at increased risk for cardiovascular disease6.

HK2: Hexokinase 2 catalyzes the first step in glucose metabolism, is of high biological relevance to COPD given the observations that (1) Whole-body glucose production is increased in patients with COPD7, (2) metabolic syndrome has been associated with COPD (and acute exacerbations of COPD)8, and (3) adipose tissue inflammation in obese COPD patients (but not cachectic) have been associated with insulin resistance.

Two-pronged approach for replication of associations with rate of decline of lung function

(i) a targeted deep-resequencing strategy to enable replication of the singleton-driven signal in the top four genes : **1,612 LHS samples falling within the same phenotype extremes**

(ii) an exome chip genotyping strategy: **2868 samples were sent to CIDR. DATA RELEASE 3/27/12**

- **Sample QC:** 2848 of the 2868 samples set = 99.3% success rate
- **Concordance:** 29 of the 30 blind duplicates with a 99.997% concordance rate (7,144,809 concordant genotypes / 7,145,016 total genotypes)
- **SNP**
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Next Steps:

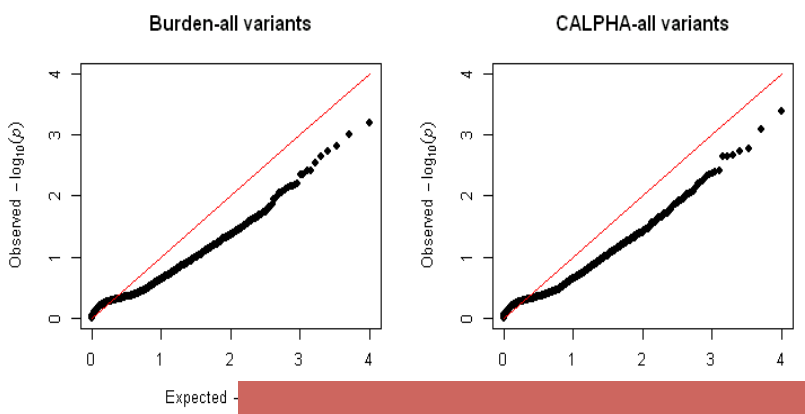
(1) Analysis the exome chip data
(2) Pending delivery of the MiSeq data on 6kb top 4 genes

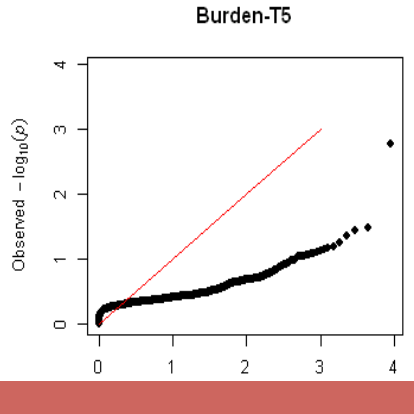
RESULTS

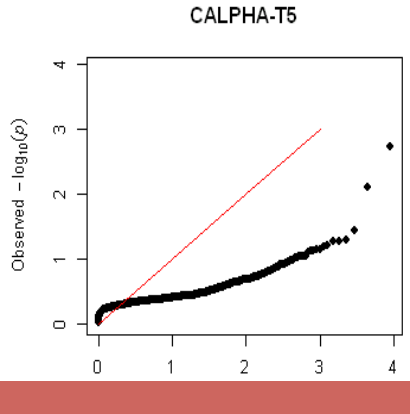
39 CASES(SSC-PAH)/ 33CONTROLS(SSC)
VARIANT N= 31,455(FILTERED AUTOSOMAL NON-SYN)
TRANSCRIPT N=9,947

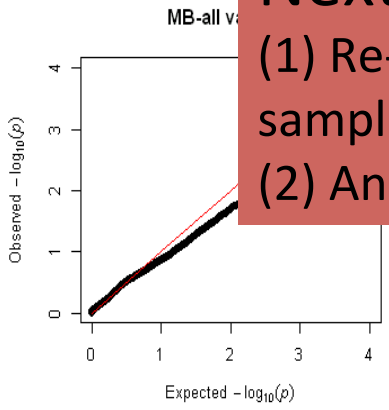
All variants

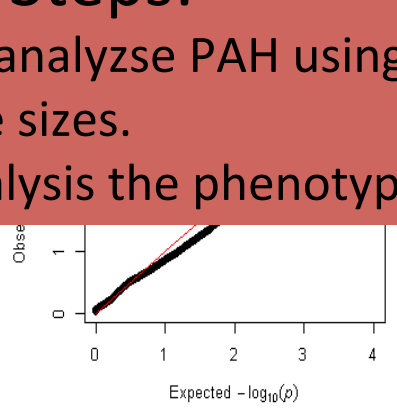
MAF<5% variants

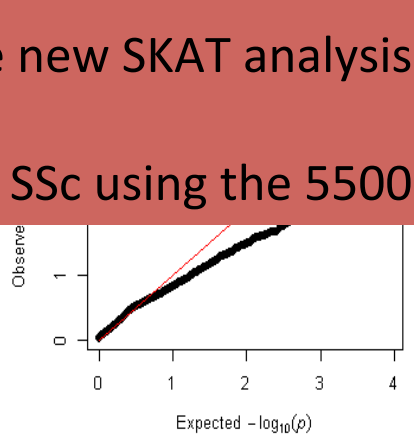


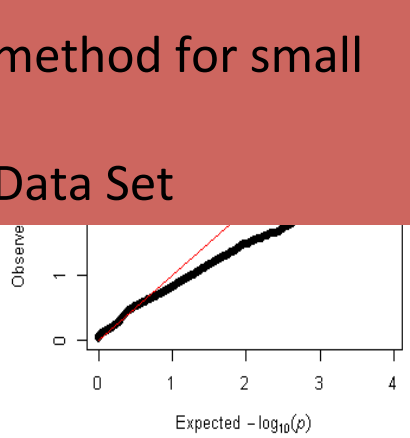










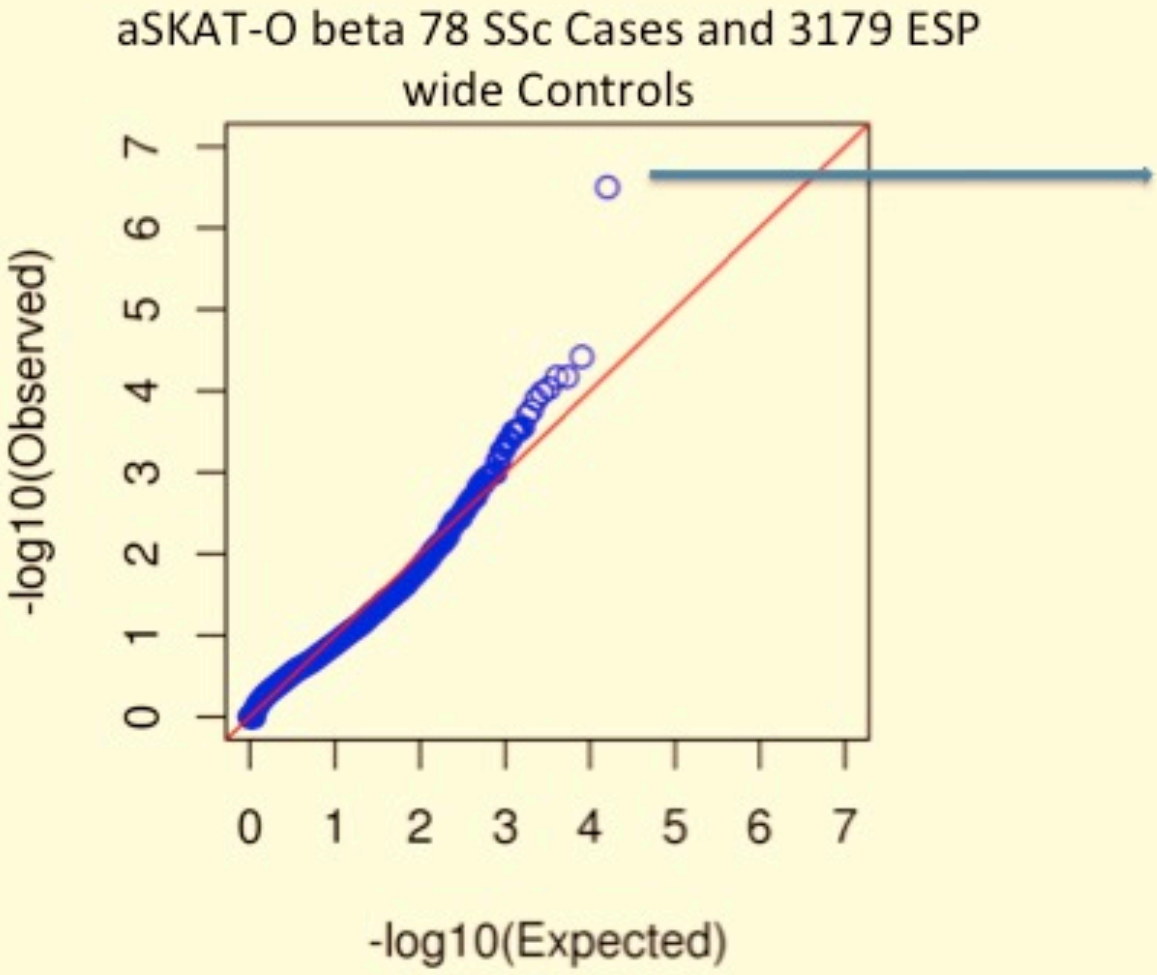


Next Steps:

(1) Re-analyzse PAH using the new SKAT analysis method for small sample sizes.

(2) Analysis the phenotype of SSc using the 5500 Data Set

Update….10:22AM March 28th



ATP8B4: ATPase Class I on Chr 15