STABILITY OF *PSEUDOMONAS AERUGINOSA* GENE TRANSCRIPTION IN CYSTIC FIBROSIS SPUTUM AND CORRELATION WITH CFQ-R RESPIRATORY SYMPTOM SCORE

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Background

Several transcriptomic analyses of *Pseudomonas aeruginosa* (*P.a.*) isolated from cystic fibrosis (CF) sputum have been presented (1-5). In four of these studies, *P.a.* was grown on artificial media, and RNA was purified, reverse transcribed, and quantified by Affymetrix® GeneChip® arrays (1-4). Real-team polymerase chain reaction (RT-PCR) was used to confirm microarray data these studies (1-4). By serially evaluating *P.a.* gene expression within the same patients, Harmer *et al.* (2) and Hoboth *et al.* (3) observed a phenotypic shift away from virulence and toward metabolic adaptation to the CF lung. Konings *et al.* (5) isolated *P.a.* RNA from CF sputum samples but still needed to quantify cDNA transcripts by RT-PCR. These authors (5) found that the expression of most *P.a.* genes did not vary according to health status, age, and gender. None of the aforementioned groups (1-5) questioned whether health-related quality of life (HRQOL) is associated with *P.a.* and/or human gene expression in CF sputum.

Herein, we used NanoString® digital multiplexed gene expression technology (6) to follow subsets of genes associated with selected *P.a.* pathways and human inflammation. We introduce an innovative workflow for efficient transcriptomic interrogation of CF sputum that does not require isolation of *P.a.* on selective media or generation of a cDNA library. We asked the following: 1) if *P.a.* behavior was similar among CF patients; 2) if *P.a.* behavior evolved within CF patients; 3) if the inflammatory milieu of the CF airway changed within patients; and 4) if *P.a.* and/or host gene expression in CF sputum correlated with CF Questionnaire-Revised (CFQ-R) domain scores (7).

1) Son MS *et al. Infect Immun* 2007; 75: 5313-24.
2) Harmer C et al. *Microbiology* 2013; 159: 2354-63.
3) Hoboth C et al. *J Infect Dis* 2009; 200: 118-30.
4) Manos J et al. *J Med Microbiol* 2008; 57: 1454-65.
5) Konings AF et al. *Infect Immun* 2013; 81: 2697-2704.
6) Kulkarni MM. *Curr Protoc Mol Biol* 2011; 94: 25B.10.1-25B.10.17.
7) Quittner AL et al. *Chest* 2005; 128: 2347-54.

Study Design

- Prospective cohort study with monthly visits for collection of sputum and patient-reported outcomes measures.
- Spirometry and anthropometric data obtained at the initial visit.

Methods

- RNA isolation: Direct-zol™ RNA MiniPrep kit (Zymo Research)
- Gene expression: nCounter® *P.a.* 75-gene custom codeset and nCounter® Human Inflammation Kit v2 (NanoString®)
- Data analysis: nSolver™ Analysis Software v1.1 (NanoString®)
- Data analysis: *gplots*, *ecodist*, and *corrplot* packages for R (R Project for Statistical Computing)

NanoString® nCounter® System

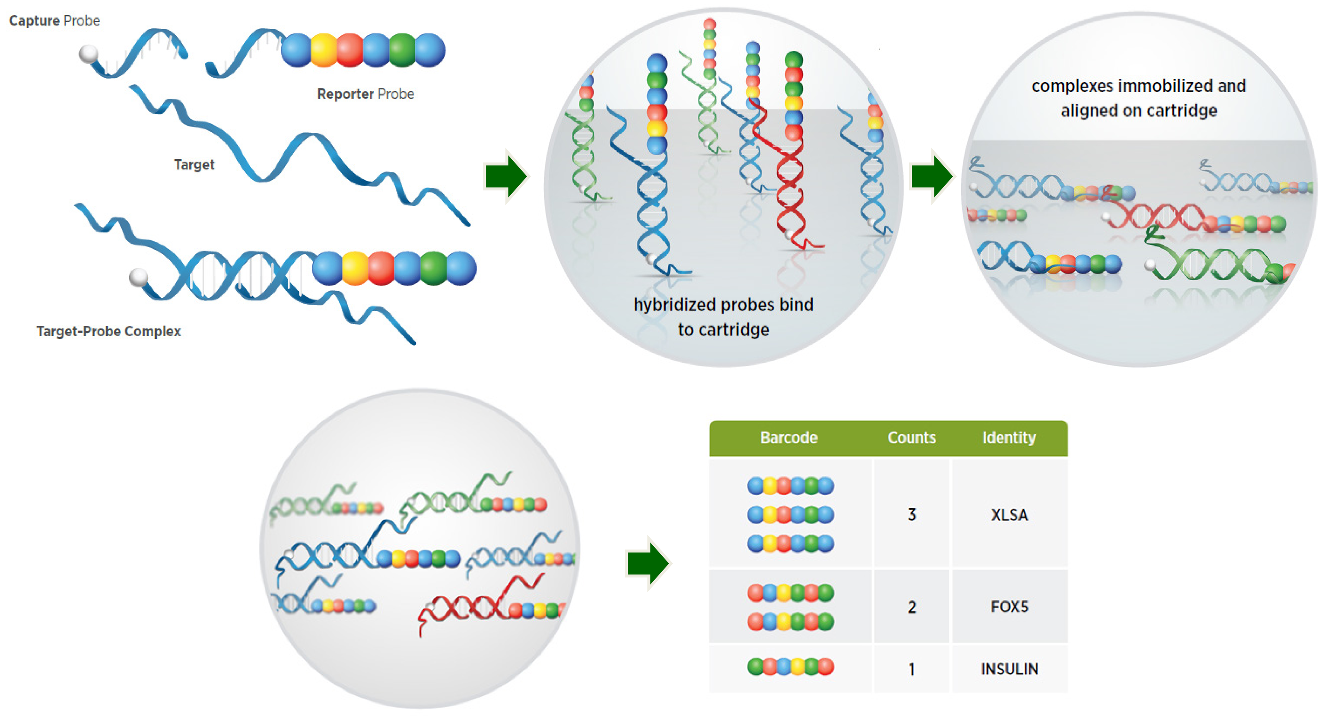

Figure 1. Each target mRNA binds to a capture probe and a reporter probe. The former immobilizes the mRNA on a cartridge. The latter uniquely identifies each transcript using a color-coded barcode system, thus facilitating quantification.

Subject Characteristics

ID	Age	Sex	BMI	FEV1%	Cycle Length†	Inhaled Antibiotic Cycle 1	2	3	4	5	6
10	32	F	20.3	55	24.3 ± 6.0	TOB	AZT	TOB	AZT	TS	TS
13	27	M	21.0	78	25.3 ± 7.9	OFF	TOB	OFF	TOB	OFF	AZT
16	24	F	22.7	66	27.7 ± 3.2	OFF	TOB	OFF	TOB	OFF	TOB
17	44	F	23.1	32	28.7 ± 5.4	COL	COL	AZT	COL	AZT	COL

† Days (mean ± SD); TOB = tobramycin inhalation solution; AZT = aztreonam lysine for inhalation; COL = inhaled colistimethate sodium; TS = trimethoprim-sulfamethoxazole

CFQ-R Domain Scores

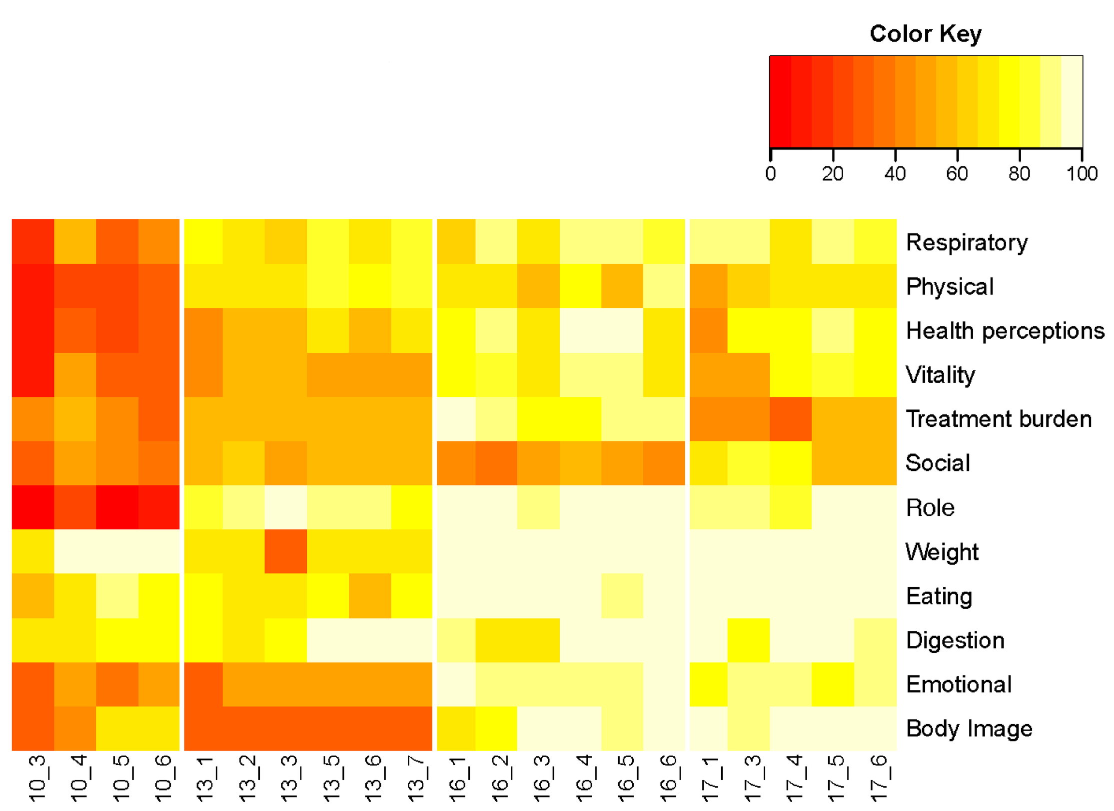

Figure 2. Hierarchical cluster analysis of serial CFQ-R domain scores. Although some within-subject variation was observed, subjects tended to have consistently higher and lower scores in specific domains over time.

P.a. Gene Ranked Abundance

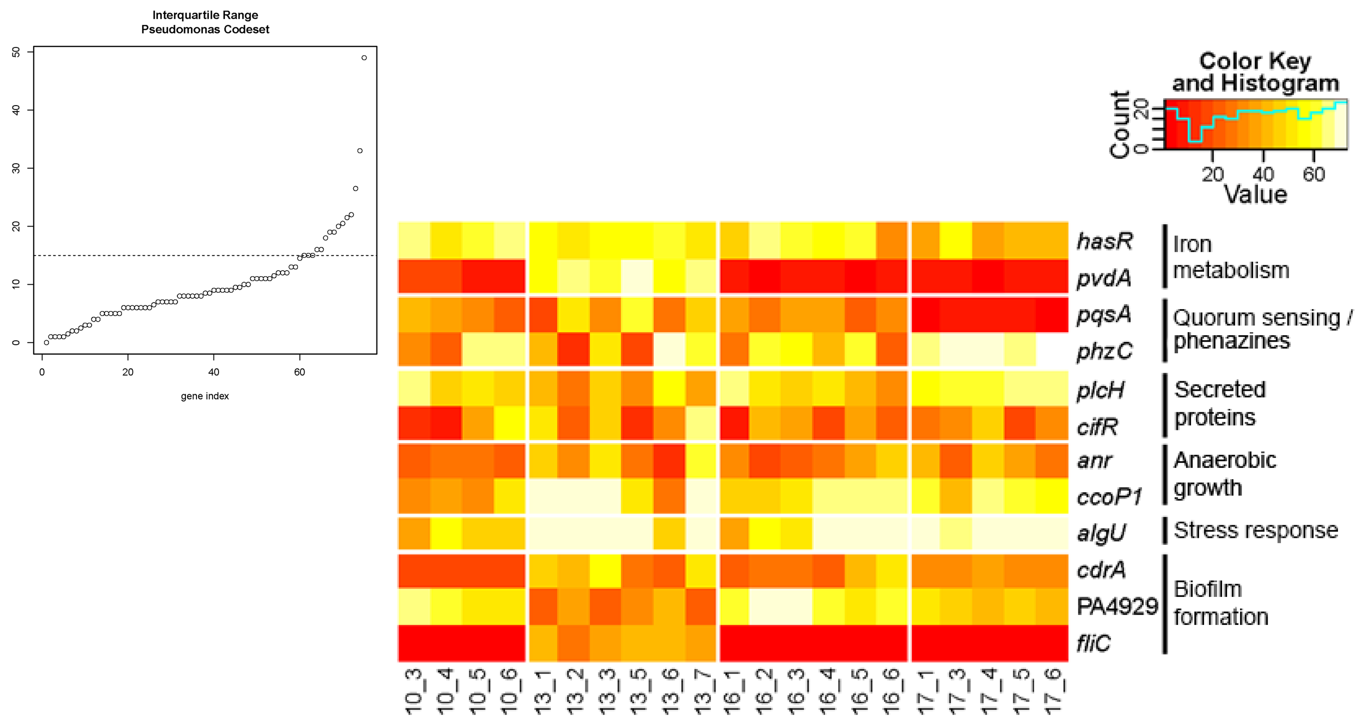

Figure 3. Hierarchical cluster analysis of *P.a.* genes with the greatest variation in ranked abundance in CF sputum (IQR >15). Expression of specific genes varied between subjects but remained largely stable within subjects.

Inflammatory Gene Ranked Abundance

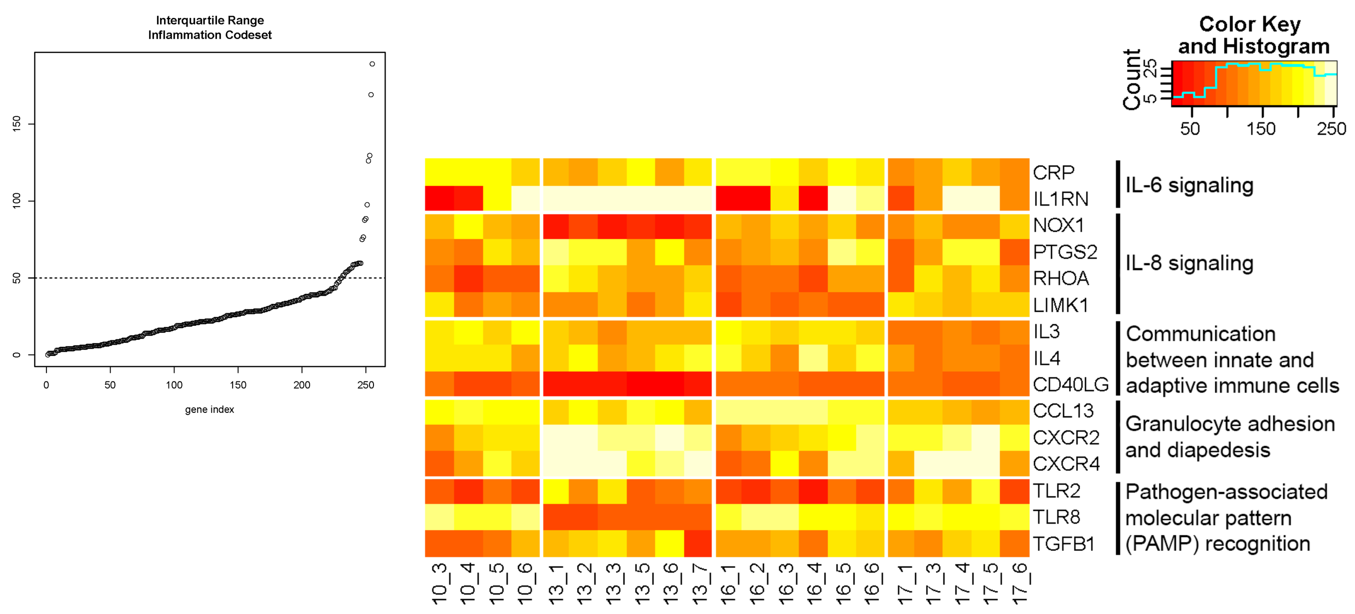

Figure 4. Hierarchical cluster analysis of human inflammatory genes with the highest variation in ranked abundance in CF sputum (IQR >50). Genes were categorized. For individual genes, abundance varied widely between subjects.

Conclusions

- Individual subjects displayed consistent scoring patterns for the 12 CFQ-R domains. Between-subject variation was observed.
- Subsets of genes for *P.a.* and human inflammation were variably abundant. These genes belonged to discrete functional classes.
- NanoString® nCounter® technology can be used to simultaneously quantify gene transcripts for *P.a.* and human inflammation in CF sputum samples.

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