Mapping atopic dermatitis and anti–IL-22 response signatures to type 2–low severe neutrophilic asthma

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Background: Transcriptomic changes in patients who respond clinically to biological therapies may identify responses in other tissues or diseases.
Objective: We sought to determine whether a disease signature identified in atopic dermatitis (AD) is seen in adults with severe asthma and whether a transcriptomic signature for patients with AD who respond clinically to anti–IL-22 (fezakinumab [FZ]) is enriched in severe asthma.
Methods: An AD disease signature was obtained from analysis of differentially expressed genes between AD lesional and nonlesional skin biopsies. Differentially expressed genes from lesional skin from therapeutic superresponders before and after 12 weeks of FZ treatment defined the FZ-response signature. Gene set variation analysis was used to produce enrichment scores of AD and FZ-response signatures in the Unbiased Biomarkers for the Prediction of Respiratory Disease Outcomes asthma cohort.
Results: The AD disease signature (112 upregulated genes) encompassing inflammatory, T-cell, T_H2, and T_H17/T_H22 pathways was enriched in the blood and sputum of patients with asthma with increasing severity. Patients with asthma with sputum neutrophilia and mixed granulocyte phenotypes were the most enriched ($P < .05$). The FZ-response signature (296 downregulated genes) was enriched in asthmatic blood ($P < .05$) and particularly in neutrophilic and mixed granulocytic sputum ($P < .05$). These data were confirmed in sputum of the Airway Disease Endotyping for Personalized Therapeutics cohort. IL-22 mRNA across tissues did not correlate with FZ-response enrichment scores, but this response signature correlated with T_H22/IL-22 pathways.
Conclusions: The FZ-response signature in AD identifies severe neutrophilic asthmatic patients as potential responders to FZ therapy. This approach will help identify patients for future asthma clinical trials of drugs used successfully in other chronic diseases. (J Allergy Clin Immunol 2021;■■■:■■■-■■■.)

Key words: *Fezakinumab, atopic dermatitis, gene set variation analysis, IL-22, severe asthma*

Abbreviations used
AD: Atopic dermatitis
ADEPT: Airway Disease Endotyping for Personalized Therapeutics
DEG: Differentially expressed gene
ES: Enrichment score
FC: Fold-change
FDR: False-discovery rate
FZ: Fezakinumab
HC: Healthy control
LT: Leukotriene
MADAD: Meta-analysis derived atopic dermatitis
MMA: Mild to moderate asthma
PNR: Potential nonresponder
PR: Potential responder
SA: Severe asthma
T2: Type 2
TAC: Transcriptome-associated cluster
U-BIOPRED: Unbiased Biomarkers for the Prediction of Respiratory Disease Outcomes

Asthma is phenotyped according to clinical treatable traits and physiological markers including eosinophilic and noneosinophilic phenotypes.[1,2] The type 2 (T2) inflammatory phenotype characterized by high expression of an IL-13–stimulated bronchial epithelial cell signature[3,4] and elevated urinary leukotriene $(LT)E_4$[5] is a molecular phenotype characterized by high eosinophilic inflammation. However, the molecular phenotypes of non-T2 inflammation remain unclear although one phenotype has been characterized by inflammasome, TNF-α, and interferon pathway activation associated with neutrophilic asthma.[3,6,7] An IL-17 phenotype characterized by neutrophilic inflammation has also been described.[8]

IL-22 belongs to the IL-10 cytokine family and is produced by T_H17 and T_H22 cells, γδ-T cells, and type 3 innate lymphoid cells as well as neutrophils.[9] Elevated bronchoalveolar lavage[10] and serum IL-22 levels[11,12] in patients with severe asthma (SA) have been reported. Neutrophil-high patients with asthma show an upregulated presence of bronchial and nasal cells staining positive for IL-22 expression.[13,14] IL-22 suppresses IFN-γ–induced proinflammatory mediator expression by human bronchial epithelial cells,[10] indicating a potential protective role in asthma, but IL-22 also enhances the proliferation and migration of human airway smooth muscle cells, which may induce airway wall remodeling.[15,16] This suggests that IL-22 could play a role in certain endotypes of asthma.

IL-22 is implicated in other chronic inflammatory diseases including atopic dermatitis (AD), a closely-related condition to asthma, often preceding it, in the atopic march.[17] Epicutaneous sensitization in mice promotes the generation of antigen-specific IL-22–producing T cells, leading to airway inflammation and airway hyperresponsiveness following allergen challenge.[18] This suggests that IL-22 may be important in the atopic march. The anti–IL-22 mAb fezakinumab (FZ) improves AD clinical scores[19] while patients with AD with high baseline IL-22 expression showed the greatest clinical response with downregulation of transcriptomic features associated with immune pathways involved in T-cell and dendritic-cell activation.[20]

The atopic march is a term used to describe the progression of allergic disease from the early presence of AD, food allergies, and rhinitis through to asthma.[21] A recent *in silico* analysis of the protein interaction networks in these diseases identified the presence of pathways contributing to the allergic multimorbidity of these diseases.[22] We hypothesized that a gene signature from patients with AD who respond to FZ will be upregulated in other chronic inflammatory diseases such as asthma. Furthermore, analysis of these "responder signatures" will select patients most likely to respond to FZ. We analyzed differentially expressed genes (DEGs) in eczematous skin lesions of IL-22–high responders between baseline and after 12 weeks of FZ treatment to obtain an FZ-response signature. This FZ signature was used to probe the transcriptomes of the lungs and blood of the Unbiased Biomarkers for the Prediction of Respiratory Disease Outcomes (U-BIOPRED) asthma cohort to identify features of subjects with asthma who may respond to FZ. The results were validated in the independent Airway Disease Endotyping for Personalized Therapeutics (ADEPT) cohort.

METHODS

Determination of AD disease and anti–IL-22 responsive signature

Full details of AD patient demographics, samples, transcriptomic analyses, and clinical response (NCT01941537) are provided elsewhere.[20] The AD disease signature was defined by DEGs identified between eczematous or lesional skin and nonlesional skin samples with a fold-change (FC) greater than or equal to 2 or less than or equal to −2 and a false-discovery rate (FDR) of less than or equal to 0.05 for the whole AD cohort. We also used a composite AD signature derived by comparing the lesional and nonlesional skin transcriptome from 4 microarray studies (MADAD [meta-analysis derived AD]).[23]

We defined an FZ treatment response signature by analysis of the lesional biopsy data of patients with AD at baseline and after 12 weeks of FZ treatment to identify DEGs (FC ≥2 or ≤−2 and FDR < 0.05).[20] Patients with high levels of IL-22 mRNA in lesional tissue at baseline had the greatest response to FZ at both the clinical and transcriptomic levels. We used DEGs from the IL-22^{high} patients with AD to derive an FZ "superresponder" signature[20] (see Table E1 in this article's Online Repository at www.jacionline.org).

Asthma cohorts

The U-BIOPRED cohort consists of nonsmoking patients with SA, smokers and ex-smokers with SA , nonsmoking patients with mild to moderate asthma (MMA), and nonsmoking healthy controls (HCs).[24] Expression profiling was performed on RNA extracted from blood cells, sputum cells, epithelial brushings, and bronchial biopsies.[8,24] Clinical characteristics and sputum and blood proteomic (SomaLogic) metadata are stored within TransMART as part of the eTRIKs project.[25] For validation, the ADEPT cohort (NCT01274507) was analyzed.[26]

Protein and other assays

The SOMAscan proteomic assay of 1129 analytes was performed on sputum supernatants (SomaLogic, Boulder, Colo).[3] The fraction of exhaled nitric oxide was measured online using an electrochemical analyzer (NIOX MINO; Aerocrine, Solna, Sweden) at an expiratory flow rate of 50 mL/s according to American Thoracic Society/European Respiratory Society guidelines.[27] Serum IgE was measured using the Thermo Fisher (Uppsala, Sweden) CAP system. Biomarker and sputum and urinary eicosanoid data were generated by multiplex analysis and mass spectrometry.[5]

Data analysis

Analysis was performed in R version 3.5.0.[28] Gene set variation analysis was run using the R Bioconductor gene set variation analysis package[29] to calculate sample-wise enrichment scores (ESs). The ES for AD disease, FZ-response, and immunologic pathway signatures was calculated for each subject across the U-BIOPRED sample compartments. We used a linear model adjusted for age and sex and used the least squares means[30] with the Tukey *P*-value adjustment method for comparisons of families of estimates (4 for cohort, 5 for granulocyte subtype, and 4 for transcriptome-associated cluster [TAC] group[3]) to analyze the ES differences between groups. Differential expression between sputum transcriptomics of subjects with eosinophilic inflammation against those with noneosinophilic inflammation and subsequent clustering revealed 3 groups. TAC1 contains patients with a high enrichment for the Woodruff T_H2-high gene signature with a very high sputum eosinophilia. The TAC2 is characterized by inflammasome-associated pathways and high sputum neutrophilia, whereas TAC3 is associated with high levels of macrophages and a mainly paucigranulocytic phenotype.[3] Visualization of the distribution of ES was performed with the ggplot2 R package.[31] The gene set variation analysis signatures are listed in Table E1.

The FZ-response signature in U-BIOPRED sputum subjects was used to categorize patients with SA as being potential responders (PRs) (n = 26; ES ≥+0.1) or potential nonresponders (PNRs) (n = 18; ES ≤−0.1) while filtering out patients with undirected ES (>+0.1 and >−0.1), MMAs, and HCs. All categorical variables were analyzed using Fisher exact test. A *t* test was used for continuous clinical variables with normal distribution (Shapiro-Wilk test *P* value > .05), whereas the Wilcoxon rank-sum test with continuity correction was used for variables with a skewed distribution.

Differential gene (for all PRs and PNRs) and protein (for those PRs and PNRs with proteomics data) expression analysis was performed using limma 3.38.3[32] for linear model fitting for each gene or protein. Empirical Bayes moderation of SEs was used to produce tables of significant DEGs and proteins. *P* values were adjusted with the Benjamini-Hochberg FDR procedure.[33] Age and sex were not confounding variables. Significantly upregulated and downregulated genes were determined by a $\log_2$ FC of greater than or equal to 1 or less than or equal to −1 and a Benjamini-Hochberg-FDR–adjusted *P* value of less than or equal to .05. Pathway enrichment analysis was performed using ReactomePA,[34] using the human Reactome ontology,[35] with *P*-value Benjamini-Hochberg-FDR adjustment and cutoff of .05.

RESULTS

AD signature in asthma

We defined an AD disease signature (Table E1) according to whether DEGs were significantly upregulated (112 DEGS, AD-UP) or downregulated (29 DEGs, AD-DOWN) between lesional and nonlesional skin, with an FC greater than or equal to 2 or less than or equal to −2 and an FDR less than or equal to 0.05 for the whole AD cohort. T-cell, T_H2, T_H17/T_H22, and general inflammatory genes were upregulated in the AD-UP signature, whereas AD-DOWN reflected lipid pathways and pathways associated with dysregulated dermal epithelial function.[20]

This signature was applied to blood (Fig 1, *A*) and sputum (Fig 1, *B*) of the U-BIOPRED cohort. The AD-UP signature ES trended with severity: significantly enriched in the blood of patients with severe, but not MMA, asthma irrespective of smoking status (Fig 1, *A*). A similar trend was seen in the sputum of patients with SA (Fig 1, *B*). When compared by sputum TACs,[3] there was an enrichment of the AD-UP signature in sputum from TAC2 (adjusted $P = 2.87 \times 10^{-6}$) subjects (Fig 1, *C*) compared with HCs. Assessment based on sputum granulocytes further highlighted the greater enrichment of the AD-UP score in granulocytic asthma (Fig 1, *D*), with a greater ES in neutrophilic (adjusted $P = 6.83 \times 10^{-5}$) and mixed granulocytic (adjusted $P = .0005$) asthma compared with HCs. The enrichment of the AD lesion signature in asthma reflects a composite of the cells within blood and sputum.

We confirmed the appropriateness of the AD-UP signature by using the previously defined MADAD-UP pooled signature (Fig 1, *E-H*). The MADAD-UP signature is a consensus disease signature of the pathologically upregulated genes that characterize AD across several studies.[23] The overlap between the AD-UP and MADAD-UP gene signatures consisted of 84 genes. This signature was enriched in both blood (Fig 1, *E*) and sputum (Fig 1, *F*) of patients with SA irrespective of smoking status, mirroring results seen in AD-UP blood. Classifying patients with asthma according to sputum molecular phenotype or according to sputum granulocytes also demonstrated enrichment of the MADAD-UP signature in TAC2 (Fig 1, *G*) and neutrophilic/mixed granulocytic subjects (Fig 1, *H*). Overall, the AD disease signature was enriched in severe neutrophilic asthma.

Derivation of an FZ-superresponder signature in AD

The FZ treatment superresponse was defined by those subjects with a good clinical response who also had a good transcriptomic response comparing lesional biopsies at baseline and after

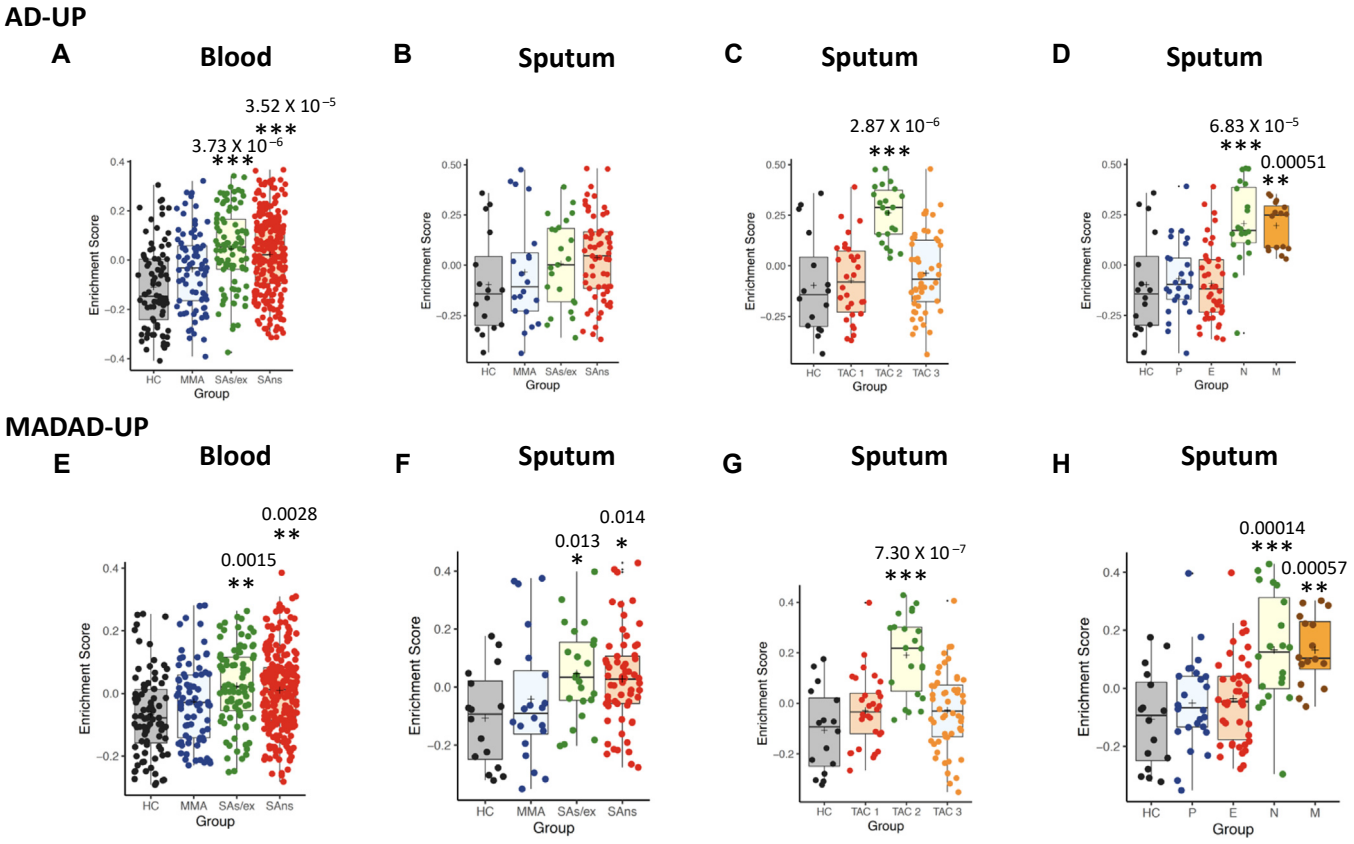

FIG 1. Gene set variation analysis showing ESs of gene signatures derived from genes upregulated (UP) in lesional vs nonlesional tissue from AD. Disease signatures are derived from either the Brunner paper (AD-UP, **A-D**) or from an AD MADAD (**E-H**). The ESs for these signatures in U-BIOPRED blood (Fig 1, *A* and *E*) and sputum (Fig 1, *B-D* and *F-H*) according to severity (Fig 1, *A, B, E,* and *F*), TAC (Fig 1, *C* and *G*), and sputum granulocyte subtype (Fig 1, *D* and *H*). Between-group adjusted *P* values are provided compared with HC values. *E*, Eosinophilic; *HC*, healthy control; *M*, mixed; *N*, neutrophilic; *P*, paucigranulocytic; *SAns*, severe asthma nonsmoker; *SAs/ex,* severe asthma smoker/ex-smoker. **P* < .05, ***P* < .01, ****P* < .001, and *****P* < .0001.

12-week FZ treatment in patients with asthma to identify the significant DEGs (FC ≥2 or ≤−2 and FDR < 0.05).[20] The highest clinical and transcriptomic effect was seen in baseline IL-22^{high} lesional tissue, and the transcriptomic changes seen in patients with a high clinical and transcriptomic response were used to generate the FZ-superresponder signature.

We identified 417 DEGs (121 upregulated and 296 downregulated by FZ) in lesional AD skin tissue biopsies from patients with the greatest clinical response to FZ at 12 weeks (Table E1). This FZ-response signature (FZ-DOWN) represents a key proportion of the AD-UP disease signatures. In particular, the AD-UP signature (112 genes) had 74 genes overlapping with the FZ-DOWN (296 genes; 25%), whereas the MADAD-UP signature (405 genes) had 196 genes overlapping with the FZ-DOWN signature (48.4%). A strong correlation existed between the AD-UP and FZ-DOWN ESs in asthmatic sputum ($R^2 = 0.8326$; $P = 2.2 \times 10^{-16}$) (see Fig E1, *A,* in this article's Online Repository at www.jacionline.org) and between MADAD-UP and FZ-DOWN ($R^2 = 0.9156$; $P = 2.2 \times 10^{-16}$) (Fig E1, *B*). The FZ-DOWN signature included pathways associated with general inflammation, T-cell, T_H2, and T_H17/T_H22 activation (see Fig E2 and Table E2 in this article's Online Repository at www.jacionline.org), which are all upregulated within the AD disease signatures. No pathways were significantly associated with FZ-UP genes although relaxing the FDR threshold identified pathways associated with epidermal signaling (see Fig E3 and Table E3 in this article's Online Repository at www.jacionline.org), which justifies the focus on the FZ-DOWN signature. To test whether the FZ-DOWN signature predicted the response in patients with AD, we examined the ES of FZ-DOWN in lesional AD baseline samples.[20] This was significantly ($P = .0496$, adjusted for age and sex) positively associated with the AD SCORing Atopic Dermatitis score after treatment.

In summary, Table E1 provides a list of all the gene signatures used in this analysis including the sets of genes upregulated (AD-UP) or downregulated (AD-DOWN) in AD, whereas Table E2 provides a list of all the pathways that the FZ-DOWN gene signature corresponds to and highlights the importance of immune pathways. Table E3 is a list of all the pathways that relate to the FZ-UP gene signature. None of these pathways was significantly enriched and are mostly skin-related.

Enrichment of the FZ superresponder signature from AD in U-BIOPRED

The FZ-DOWN signature was significantly enriched in the blood of U-BIOPRED patients with SA (adjusted $P < .05$) (Fig 2, *A*) despite the wide variability in ESs, which may reflect the

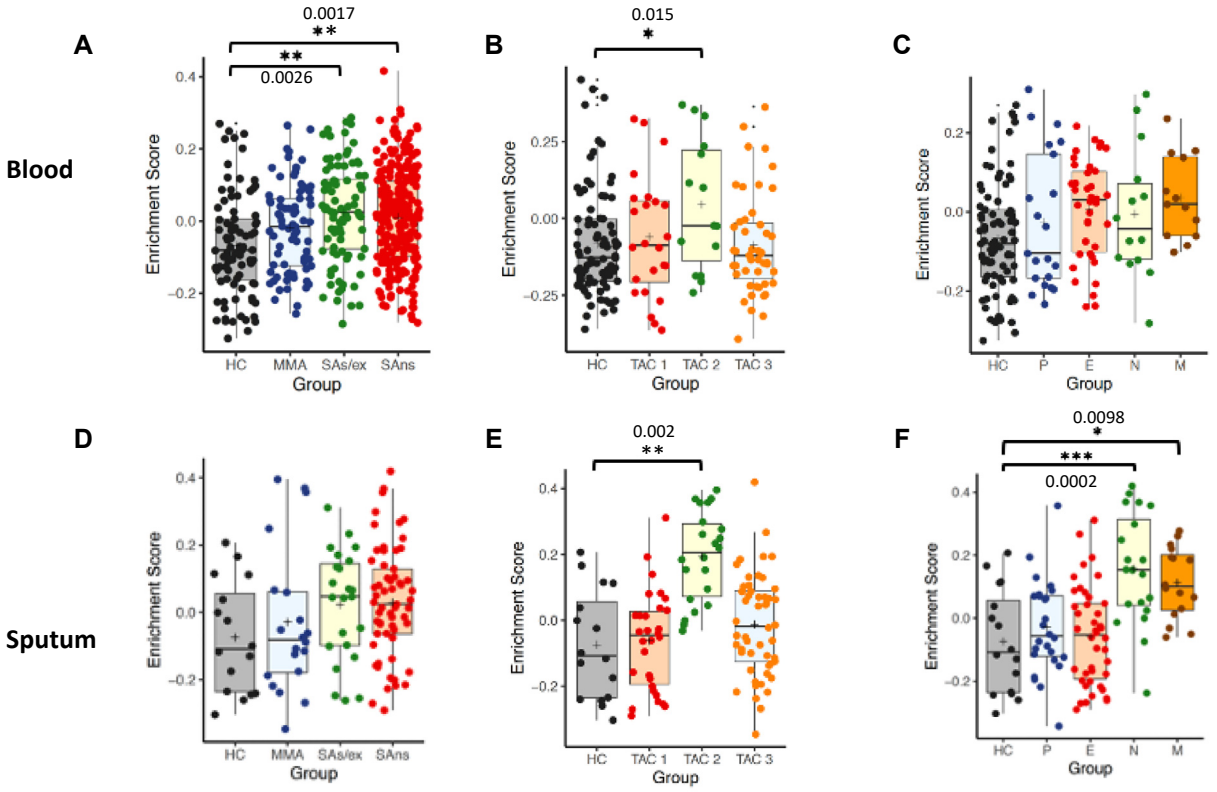

FIG 2. Gene set variation analysis showing ESs of FZ treatment response signatures of downregulated genes (FZ-DOWN) in lesional vs nonlesional tissue from AD. ESs for AD-UP signatures are given for U-BIOPRED blood (**A-C**) and sputum (**D-F**) according to asthma severity (Fig 2, *A* and *D*), TAC (Fig 2, *B* and *E*), and granulocyte subtype (Fig 2, *C* and *F*). Between-group adjusted *P* values are provided compared with HC values. *E*, Eosinophilic; *HC*, healthy control; *M*, mixed; *N*, neutrophilic; *P*, paucigranulocytic; *SAns*, severe asthma nonsmoker; *SAs/ex*, severe asthma smoker/ex-smoker. **P* < .05, ***P* < .01, ****P* < .001, and *****P* < .0001.

different types of immune cells found in blood and lesional tissue. The skin contains a mixture of epithelial cell–like and immune cells, but the enrichment observed in blood may indicate detection of the immune components.

The FZ-DOWN signature was significantly enriched in the blood of TAC2 patients (adjusted $P = .015$; Fig 2, *B*). The response in blood when subjects were stratified according to sputum granulocytes was variable, and although there was a trend toward enrichment in asthma subtypes, this did not reach significance (Fig 2, *C*). There was a greater degree of enrichment in sputum samples compared with blood (compare Fig 3, *A-C*, with Fig 3, *D-F*). The ES for FZ-DOWN had a stepwise association with severity and was highly enriched in TAC2 patients (adjusted $P = .002$; Fig 2, *E*), and in patients with neutrophilic (adjusted $P = .0002$; Fig 2, *F*) and mixed granulocytic (adjusted $P = .0098$; Fig 2, *F*) asthma compared with HCs. The good correlation between the TAC2 signature and the FZ-DOWN signature in sputum ($P < 2.2 \times 10^{-16}$; $r = 0.784$) was not due to overlapping signatures because only 3 genes were common between the 2 gene sets—CASP4, KCNJ15, and SAMSN1. Importantly, we were able to show that the AD-UP and MADAD-UP (Fig 3, *A*) and the FZ-DOWN (Fig 3, *B*) signatures were also enriched within the sputum neutrophilic (adjusted $P < .05$) and mixed granulocytic patients within the ADEPT cohort (Fig 3, *A* and *B*).

To ensure against a confounding effect of tissue heterogeneity, we removed the 4 skin-specific genes identified by comparing the FZ-DOWN signature with a published skin transcriptomic profile.[36] There were 4 overlapping genes (WFDC12, TYR, S1PR5, LYPD5), and removal of these 4 genes from the FZ-DOWN signature had minimal effect on the analysis (see Fig E4 in this article's Online Repository at www.jacionline.org).

Because the FZ-DOWN signature was associated with neutrophilic asthma, we checked whether this and the AD disease signatures correlated with 3 neutrophil signatures from the Human Cell Atlas,[37] an immune cell gene-signature database[38] and a T_H17 signature[39] that consists of genes for neutrophil chemoattractants (CXCL1, CXCL2, CXCL3, CXCL8, and CFS3). We observed a high correlation between FZ-DOWN ES and neutrophil signature ES and also AD disease signature ES and neutrophil signature ES, indicating that the disease signatures reflected tissue neutrophilia. In particular Pearson correlation between the FZ-DOWN ($P = 1.25 \times 10^{-9}$; $r = 0.519$), AD-UP ($P < 2.2 \times 10^{-16}$; $r = 0.754$), and the MADAD-UP ($P < 2.2 \times 10^{-16}$; $r = 0.684$) signatures was very significantly correlated with the immune cell database neutrophil signature. In addition, the FZ-DOWN ($P < 2.2 \times 10^{-16}$; $r = 0.691$), AD-UP ($P = 4.479 \times 10^{-7}$; $r = 0.441$), and the MADAD-UP ($P = 4.304 \times 10^{-7}$; $r = .442$) signatures were significantly correlated with the Human Cell Atlas neutrophil signature.

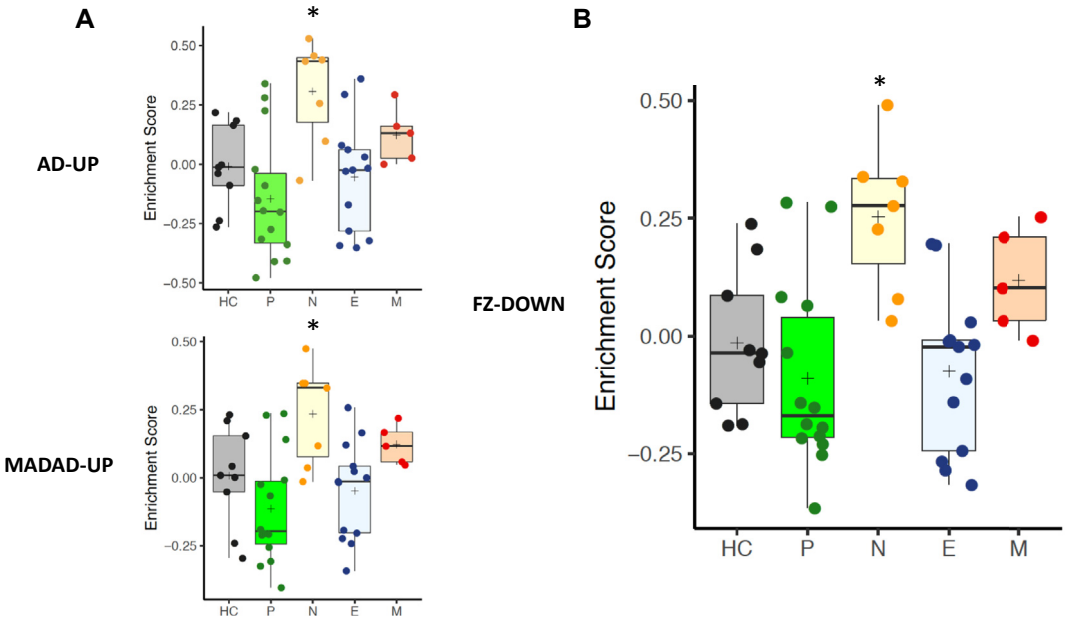

FIG 3. Gene set variation analysis showing ESs of gene signatures derived from genes upregulated (UP) in lesional vs nonlesional tissue from AD in the ADEPT cohort by granulocytic subtype. Disease signatures are derived from either the Brunner paper (AD-UP, **A**, *upper panel*) or from an AD MADAD (Fig 3, *A*, *lower panel*). ES of the FZ-DOWN signature obtained from lesional vs nonlesional tissue after 12-week treatment (**B**). Between-group adjusted *P* values are provided compared with HC values. *E*, Eosinophilic; *HC*, healthy control; *M*, mixed; *N*, neutrophilic; *P*, paucigranulocytic. *$P < .05$.

However, neutrophil levels in the skin were not significantly reduced after FZ treatment (see Fig E5 in this article's Online Repository at www.jacionline.org), which suggests that despite neutrophil genes contributing to the AD disease signature and some neutrophil genes being present in the FZ-response signature, the FZ-response phenomenon is unlikely to be driven by neutrophil levels alone. This is corroborated by the positive but nonsignificant correlation between sputum neutrophils and sputum IL-22 protein in U-BIOPRED subjects ($P = .0699$; $r = 0.184$). We also examined the correlation between sputum neutrophils and the FZ-DOWN signature in the validation ADEPT cohort and found no significant correlation (% segmented neutrophils; $P = .911$; $r = 0.0186$).

Clinical features of PRs and PNRs in U-BIOPRED

We next examined whether the FZ-DOWN signature was associated with a specific subset of patients with SA as the most clinically relevant group. Highly enriched patients (PRs) were compared with those least-enriched (PNRs) for the FZ-DOWN signature (see Fig E6 and Table E4 in this article's Online Repository at www.jacionline.org). The enrichment score of the FZ-DOWN signature in sputum was used to categorize patients with SA as being PRs (n = 26; ES ≥ +0.1) or PNRs (n = 18; ES ≤ −0.1) while filtering out patients with an undirected ES (<+0.1 and >−0.1), MMAs, and HCs. The clinical comparison revealed that PRs had more frequent LABA use and significantly elevated sputum neutrophils and lower sputum eosinophils and macrophages in addition to lower IgE levels in contrast to PNRs (Table I). Furthermore, PRs had lower levels of plasma eotaxin-3 and serum IL-13 biomarkers as measured by Luminex or MSD analysis. PRs also had elevated sputum levels of 11-dehydro-TXB_2, 5-HETE, and LTB_4 ($P = .0526$) but lower LTE_4, reflecting the neutrophilic and low eosinophilic nature of the PR population (Table II).

In a linear model of asthmatic sputum FZ-DOWN ES and medication usage, corrected for age, sex, and body mass index, we found no significant association between FZ-DOWN ES and oral corticosteroid use ($P = .702$). However, we did find a significant association between FZ-DOWN ES and LABA use ($P = .0243$) where FZ-DOWN ES was elevated in the twice-daily LABA use group (reflecting severity of disease, linear model estimate = 0.112) and least in the group not taking LABA at all (mildest subjects, linear model estimate = −0.117).

DEGs between PR and PNR patients with SA

We performed DEG analysis between PR and PNR patients and identified 431 up and 19 down sputum DEGs that were significant with a $\log_2$ FC of above 1 or below −1, respectively. These are reported in Table E5 in this article's Online Repository at www.jacionline.org. ReactomePA pathway analysis on the up DEGs indicates a strong neutrophilic component with neutrophil degranulation, cytokine and chemokine receptor, and Toll-like receptor signaling as well as IL-10 and interferon pathways being highly enriched in PR subjects (Fig 4; see Table E6 in this article's Online Repository at www.jacionline.org). The IL-33 receptor (IL1RL1, ST2) was greatly downregulated in the PR group.

Sputum proteomic enrichment of FZ-DOWN signature

We then selected PRs and PNRs who had SomaLogic sputum proteomics data available (n = 32) (see Table E7 in this article's Online Repository at www.jacionline.org). Differential protein analysis on the sputum SomaLogic data confirmed a strong neutrophilic component (Table III). Significantly upregulated

TABLE I. Clinical differences of PRs vs PNRs to FZ in U-BIOPRED

Characteristic	FZ PRs	FZ PNRs	*P* value
Total (n)	26	18	
Age (y)	51.8 ± 12.7	55.3 ± 14	NS
BMI	28 ± 4.65	26.3 ± 3.39	NS
Sex: female, n	16	9	NS
SA, nonsmokers, n	18	13	NS
SA, smokers/ex-smokers, n	8	5	NS
Severe exacerbation in previous year	2.27 ± 2.38	1.72 ± 2.02	NS
Nasal polyps, n	8	6	NS
Eczema, n	8	7	NS
Allergic rhinitis, n	9	4	NS
Nonallergic rhinitis, n	5	3	NS
Gastroesophageal reflux, n	12	7	NS
Hay fever, n	11	5	NS
Positive atopic status, n	11	6	NS
ACQ-5 score	2.44 ± 1.23	1.79 ± 1.31	NS
AQLQ score	4.35 ± 1.22	4.98 ± 1.41	NS
HADS score	12.3 ± 8.16	10.5 ± 8.91	NS
SNOT score	31.2 ± 18.2	22.6 ± 10.8	NS
FEV_1 (% predicted)	63.7 ± 24.3	67.2 ± 17.5	NS
FVC (% predicted)	86.9 ± 20.4	95.3 ± 17.4	NS
FEV_1/FVC	59.3 ± 13.1	57.2 ± 8.79	NS
FENO (ppb)	35 ± 33	54.7 ± 46.9	NS
Serum IgE (IU/L)	204 ± 358	332 ± 294	.02
Blood eosinophil ($/10^{-9}$L)	0.277 ± 0.155	0.401 ± 0.305	NS
Blood neutrophil ($/10^{-9}$L)	4.93 ± 1.93	5.41 ± 2.44	NS
Blood lymphocyte ($/10^{-9}$L)	2.12 ± 0.936	2.1 ± 0.9	NS
Blood monocyte ($/10-9^{L}$)	0.634 ± 0.278	0.581 ± 0.222	NS
Sputum neutrophils (%)	75.7 ± 16.6	35.6 ± 18	2.26×10^{-08}
Sputum eosinophils (%)	3.9 ± 5.55	30.4 ± 26.5	.0009
Sputum lymphocyte (%)	1.5 ± 1.6	1.46 ± 1.26	NS
Sputum macrophage (%)	18.9 ± 14.4	32.5 ± 20.8	.025
Sputum mast cell (%)	0.0346 ± 0.087	0.0333 ± 0.101	NS
Oral corticosteroid use daily, n	12	9	NS
LABA use twice a day, n	12	2	.039

ACQ, Asthma Control Questionnaire; *AQLQ*, Asthma Quality of Life Questionnaire; *BMI*, body mass index; *FVC*, forced vital capacity; *FENO*, fractional exhaled nitric oxide; *HADS*, Hospital Anxiety and Depression Scale; *ICS*, inhaled corticosteroid; *LABA*, long-acting β-agonist; *NS*, not significant; *SNOT*, SinoNasal Outcome Test.
Data shown as mean ± SD.

sputum proteins included the neutrophil modulator sialic acid–binding immunoglobulin-type lectins 9, the neutrophil serine proteases cathepsin G and azurocidin involved in neutrophil degranulation and microbial killing, B7_H2, which is a costimulatory ligand for CD28, IL-6, which is involved in neutrophilic asthma and increased differentiation of T_H17 cells, and oxidized low-density lipoprotein receptor 1, which is involved in tissue remodeling. These proteins together with the enhanced expression of neutrophil degranulation products implicate neutrophil activation as being a key component of subjects with asthma who are highly enriched for the FZ-DOWN signature.

FZ-DOWN signature markers in blood

We then selected PRs and PNRs who had blood proteomics data available (n = 42) (see Table E8 in this article's Online Repository at www.jacionline.org). Differential protein analysis on blood SomaLogic data (see Table E9 in this article's Online Repository at www.jacionline.org) defined potential FZ responders from nonresponders as possessing lower blood IgE and a trend toward elevated expression of the neutrophil modulator sialic acid–binding immunoglobulin-type lectins 9 and interferon-inducible T-cell alpha chemoattractant (I-TAC) as seen in the sputum proteomics analysis.

IL-22 pathway and protein correlate with FZ-DOWN enrichment

In AD skin,[20] IL-22 gene expression alone predicts the response to FZ. However, IL-22 gene expression was not enriched in blood (see Fig E7, *A*, in this article's Online Repository at www.jacionline.org) or sputum according to asthma severity (Fig E7, *B*) or in TAC2 patients with asthma (Fig E7, *C*). There was no correlation between FZ-DOWN and IL-22 gene expression in blood (Fig E7, *D*), sputum (Fig E7, *E*), bronchial brushings (Fig E7, *F*), or nasal brushings (Fig E7, *G*).

In contrast, the ES of the T_H22/IL-22 signature was significantly correlated with FZ-DOWN ES in asthmatic sputum ($P = 4.31 \times 10^{-14}$; $r = 0.656$) (Fig 5, *A*), bronchial brushings ($P < 2.2 \times 10^{-16}$; $r = 0.753$) (Fig 5, *B*), nasal brushings ($P = 8.53 \times 10^{-13}$; $r = 0.755$) (Fig 5, *C*), and blood ($P = 5.06 \times 10^{-6}$; $r = 0.223$). The T_H22/IL-22 signature (Table E1) consists of 16 genes including IL-22 itself and the T_H22-specific marker CCR10.[20] Pathway analysis identified several significantly enriched pathways including "IL22 Induces Keratinocyte Proliferation in Psoriasis," "Interleukin-19, 20, 22, 24 Homo sapiens R-HSA-8854691," and "IL-the 17 signaling pathway."

Importantly, sputum IL-22 protein was significantly enriched in patients with TAC2 asthma compared with those with TAC1

TABLE II. Molecular marker differences of PRs vs PNRs to FZ in the U-BIOPRED patients with SA

Biomarker	FZ PRs	FZ PNRs	*P* value
α1 microglobulin (pg/mL) Luminex (serum)	6,120 (2,210)	7,500 (2,390)	NS
C5a (pg/mL) Luminex (serum)	50.8 (33.4)	38.9 (21.6)	NS
CD30 (pg/mL) Luminex (serum)	38.9 (17.1)	42.5 (14.7)	NS
CD40L (pg/mL) Luminex (serum)	4,420 (1,990)	5,210 (2,300)	NS
DPPIV (pg/mL) Luminex (serum)	98,500 (48,100)	91,500 (24,300)	NS
Galectin 3 (pg/mL) Luminex (serum)	5,550 (2,050)	5,770 (1,470)	NS
IL-18 (pg/mL) Luminex (serum)	247 (152)	234 (73.8)	NS
IL-1α (pg/mL) Luminex (serum)	35.5 (9.95)	36.2 (6.12)	NS
IL-6Rα (pg/mL) Luminex (serum)	10,600 (2,450)	10,900 (2,020)	NS
LBP (pg/mL) Luminex (serum)	2,110,000 (891,000)	1,820,000 (668,000)	NS
Lumican (pg/mL) Luminex (serum)	131,000 (37,000)	136,000 (25,300)	NS
MCP4 (pg/mL) Luminex (serum)	142 (44.7)	168 (71.2)	NS
MMP3 (pg/mL) Luminex (serum)	21,400 (18,300)	24,500 (17,900)	NS
RAGE (pg/mL) Luminex (serum)	1,260 (414)	1,320 (382)	NS
Serpin E1 (pg/mL) Luminex (serum)	95,000 (30,400)	97,600 (19,900)	NS
SHBG (pg/mL) Luminex (serum)	3640,000 (2,840,000)	4,780,000 (4,670,000)	NS
CCL17 (pg/mL) MSD (plasma)	77.5 (70.8)	134 (120)	NS
CCL22 (pg/mL) MSD (plasma)	796 (316)	866 (218)	NS
EOTAXIN (pg/mL) MSD (plasma)	118 (60.1)	140 (67.6)	NS
EOTAXIN3 (pg/mL) MSD (plasma)	15 (15.4)	72.4 (130)	.00097
IFN-γ (pg/mL) MSD (plasma)	12.2 (12.8)	7.44 (6.18)	NS
IL-6 (pg/mL) MSD (plasma)	1.21 (1.01)	0.804 (0.335)	NS
IL-8 (pg/mL) MSD (plasma)	6.02 (9.75)	3.78 (1.86)	NS
IP10 (pg/mL) MSD (plasma)	386 (250)	305 (183)	NS
MCP1 (pg/mL) MSD (plasma)	117 (36.8)	119 (38.4)	NS
MIP1β (pg/mL) MSD (plasma)	56.1 (18.1)	63.5 (29.8)	NS
TNF-α (pg/mL) MSD (plasma)	1.84 (0.483)	1.94 (0.632)	NS
CCL18 (pg/mL) IMPACT serum	169 (63.3)	228 (106)	NS
IL-13 (pg/mL) IMPACT serum	0.608 (0.494)	0.942 (0.384)	.0074
IL-17A (pg/mL) SINGULEX serum	0.58 (0.381)	0.455 (0.258)	NS
Periostin (ng/mL) ELECSYS serum	51.2 (19.5)	54.2 (16.5)	NS
hCRP (mg/L)	6.29 (11)	1.69 (1.33)	NS
11-DehydroTXB$_2$ (ng/mL) urine	13.9 (8.96)	14.2 (10.5)	NS
2,3 Dinor-11β PGF2α (ng/mL) urine	73.8 (30.9)	94.8 (86)	NS
2,3 Dinor 8isoPGF2α (ng/mL) urine	244 (137)	296 (319)	NS
2,3 Dinor TXB$_2$ (ng/mL) urine	68.1 (46.6)	52.2 (41.3)	NS
8,12 IsoPGF2α (ng/mL) urine	386 (240)	430 (408)	NS
8 IsoPGF2α (ng/mL) urine	29.3 (11.8)	32.3 (19.6)	NS
LTE$_4$ (ng/mL) urine	9.28 (8.35)	10 (6.13)	NS
PGE2 (ng/mL) urine	20.2 (23.2)	18.5 (14.8)	NS
PGF2α (ng/mL) urine	132 (102)	130 (75.1)	NS
Tetranor PGDM (ng/mL) urine	299 (115)	305 (257)	NS
TetranorPGEM (ng/mL) urine	1,180 (1190)	1,030 (539)	NS
11 DehydroTXB$_2$ (pg/mL) sputum	231 (283)	63.4 (23.9)	.00438
12-HETE (pg/mL) sputum	1,470 (1300)	1,980 (1410)	NS
15-HETE (pg/mL) sputum	4,490 (6900)	7,180 (9130)	NS
5-HETE (pg/mL) sputum	1,570 (1500)	964 (1630)	.0322
6-KetoPGF1α (pg/mL) sputum	58.6 (27.1)	53.7 (23.6)	NS
LTB$_4$ (pg/mL) sputum	801 (756)	774 (1540)	NS
LTE$_4$ (pg/mL) sputum	319 (372)	763 (1030)	.0312
PGD2 (pg/mL) sputum	269 (317)	174 (159)	NS
PGE2 (pg/mL) sputum	390 (363)	202 (135)	NS
Tetranor PGDM (pg/mL) sputum	66 (57.4)	54.8 (51.9)	NS
Tetranor PGEM (pg/mL) sputum	76.3 (49.1)	67.6 (53.3)	NS

C5a, Complement component 5a; *CCL17*, C-C motif chemokine ligand 17; *CD30*, cluster of differentiation 30; *CD40L*, CD40 ligand; *DPPIV*, dipeptidyl peptidase-4; *hCRP*, human C-reactive protein; *IP10*, interferon gamma-induced protein 10; *LBP*, lipopolysaccharide binding protein; *LTE$_4$*, leukotriene E4; *MCP1*, monocyte chemoattractant protein-1; *MCP4*, monocyte chemoattractant protein 4; *MIP1β*, macrophage inflammatory protein 1b; *MMP3*, matrix metalloproteinase-3; *NS*, not significant; *PGDM*, prostaglandin D metabolite; *PGEM*, tetranor-prostaglandin E metabolite; *PGF2a*, prostaglandin F2 alpha; *RAGE*, receptor for advanced glycation endproducts; *SHBG*, sex hormone-binding globulin; *TNFa*, tumor necrosis factor alpha; *TXB$_2$*, thromboxane B2; *12-HETE*, 2-hydroxyeicosatetraenoic acid.

asthma ($P = .0112$), and there was a significant correlation between sputum IL-22 protein expression and the FZ-DOWN ES when controlled for age, sex, and body mass index ($P = .0360$; $r = 0.133$) (Fig 5, *D*). IL-22 protein in sputum also significantly correlated with FZ-DOWN ES in nasal brushings for all subjects ($P = .0443$; $r = 0.423$).

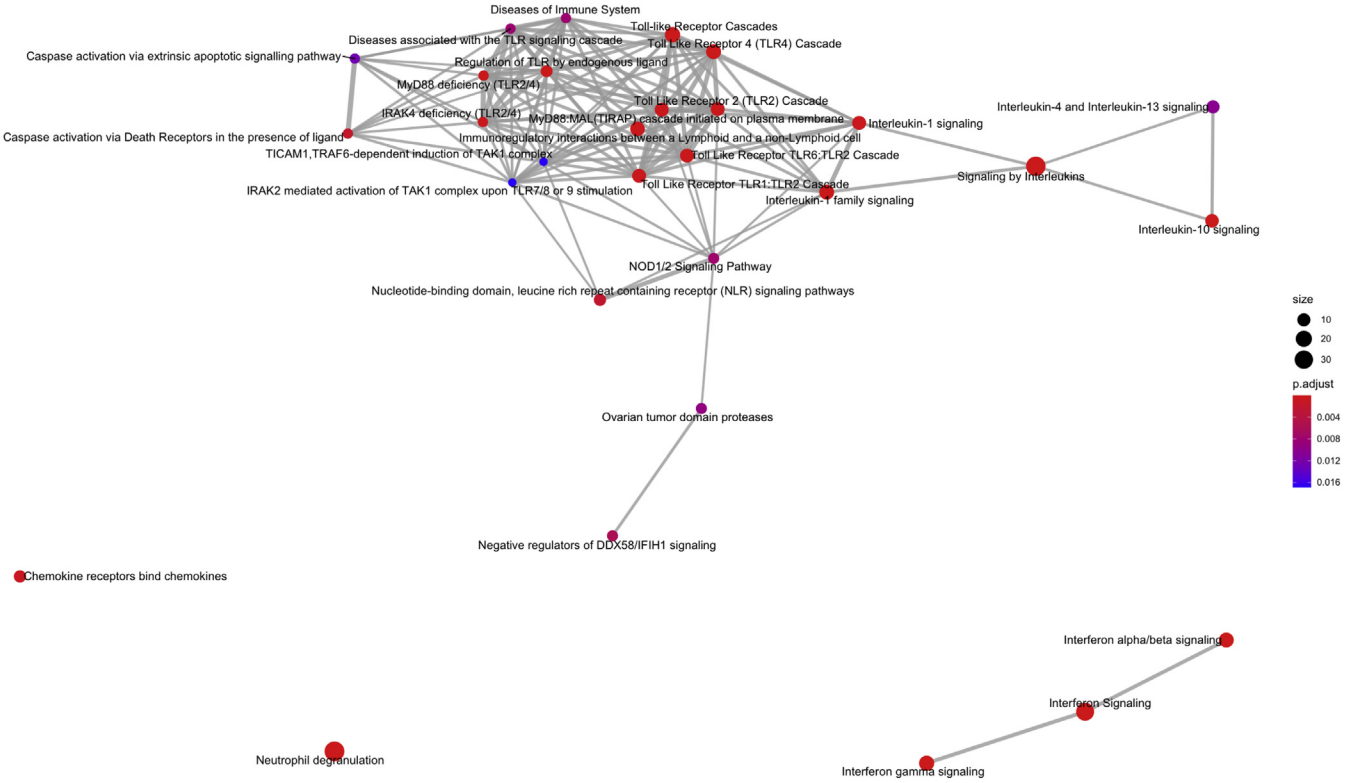

FIG 4. Protein pathway analysis using ReactomePA of DEGs (FDR < 0.05) that distinguish patients with asthma highly enriched (PRs) for the FZ-response signature (FZ-DOWN) from those poorly enriched (PNRs) for this signature.

DISCUSSION

We demonstrate that an AD disease signature was enriched in severe neutrophilic asthma in both the U-BIOPRED and ADEPT asthma cohorts and that these subjects were also highly enriched for a gene signature indicative of a superresponse to FZ. Pathway analysis indicated that the AD-UP disease signature and the FZ-DOWN response signature were a composite of T_H1, T_H2, T_H17, T_H22, and general inflammatory processes and that sputum proteins linked with a potential FZ response in asthma were associated with neutrophil recruitment and activation. The FZ superresponse signature did not correlate with IL-22 gene expression itself although there was a good correlation with the T_H22/IL-22 gene signature in nasal and bronchial brushings. Sputum IL-22 protein correlated significantly with FZ-DOWN. Repurposing transcriptomic data that define a treatment response across therapeutic areas may aid the stratification of patients for future clinical trials.

Early transcriptomic analysis of skin samples from subjects with psoriasis and AD identified neutrophil chemoattractant genes as being highly expressed in both AD and psoriatic skin lesions.[40] Furthermore, neutrophil elastase staining is elevated in lesional compared with nonlesion skin in patients with AD but to a much lesser extent than seen in patients with psoriasis. This enhanced neutrophilia in AD may reflect concurrent infection with *Staphylococcus aureus* infection.[41] Enhanced neutrophilia may reflect an enhanced T_H1/T_H17 drive.

The T_H2/T_H22 pathway is the major pathway in AD as recently confirmed using single-cell RNA sequencing.[42] This is seen across all age groups; however, an enrichment of T_H1/T_H17 genes is seen in lesional compared with nonlesional skin in adults.[43] Indeed, the usual T_H2/T_H22 drive in AD is skewed toward a T_H1/T_H17 phenotype with increasing age[44] and severity of disease. For example, enhanced T_H1/T_H17 mediator expression is reported in the blood of patients with AD with severe but not mild disease.[45] Importantly, there was a good correlation between $T_H2/T_H22/T_H1/T_H17$ gene and protein expression profiles in lesional and nonlesional AD samples.[46]

Severe asthmatic PRs to FZ had neutrophilic or mixed granulocytic asthma, poor lung function, and a low asthma quality of life despite frequent LABA use. These subjects also had lower serum IgE levels but with relatively greater atopic disposition, in contrast to subjects with T2 eosinophilic asthma (≥300 cells/μL), suggesting that an anti–IL-22 intervention may be targeted to non-T2 patients with asthma with low IgE as opposed to those with a high IgE neutrophilic phenotype.[14,47] Sex, body mass index, and age did not affect the enrichment of the FZ response signature. Comparison of biomarkers between PRs and PNRs indicated that PR subjects had elevated levels of 11-dehydro-TXB_2, 5-HETE, and LTB_4 although the latter did not quite reach significance. Leukotrienes are formed via a 5-LOX–dependent process in which arachidonic acid is converted to the unstable epoxide intermediate LTA_4, which can then be converted by either LTC_4 synthase to form the cysteinyl-leukotrienes or via LTA_4-hydrolase to form LTB_4. Neutrophils have known LTA_4-hydrolase activity, and sputum neutrophils have been previously reported to produce LTB_4.[48] Accordingly, the elevated sputum LTB_4 levels in combination with the lower LTE_4 levels among PR subjects collectively point toward a specific elevation of

TABLE III. Top and bottom 20 differentially expressed sputum proteins that differentiate U-BIOPRED asthmatic FZ PRs from PNRs defined from asthma sputum GSVA FZ-response signature ES that had sputum proteomic data available (see Table E7 and Fig E4)

Upregulated				
Gene symbol	**Log_2 FC**	**FC**	***P* value**	**FDR-BH–adjusted *P* value**
Siglec_9	2.04	4.11	.0066	.1928
Hemoglobin	1.98	3.96	.01807	.2210
PSA1	1.75	3.37	.01409	.2059
Cathepsin_G	1.70	3.25	.00048	.1368
Carbonic_anhydrase_I	1.45	2.74	.00081	.1370
SRCN1	1.43	2.70	.00702	.1928
Azurocidin	1.40	2.65	.00251	.1661
PLCG1	1.30	2.46	.01068	.2059
Resistin	1.29	2.45	.09375	.3715
Factor_I	1.28	2.44	.14821	.4261
IL_6	1.28	2.43	.00390	.1869
B7_H2	1.19	2.29	.04214	.2830
Ferritin	1.18	2.27	.0127	.2059
IP_10	1.15	2.23	.01319	.2059
Elastase	1.08	2.12	8.53×10^{-05}	.0959
Transferrin	1.06	2.09	.16571	.4476
OLR1	1.02	2.03	.00309	.1735
I_TAC	0.99	1.99	.04652	.2875
Granzyme_B	0.99	1.98	.03350	.2599
Esterase_D	0.97	1.96	.06937	.338
Downregulated				
Gene symbol	**Log_2 FC**	**FC**	***P* value**	**FDR-BH–adjusted *P* value**
a2_Antiplasmin	−1.28	0.40	.02414	.2441
Fucosyltransferase_3	−1.29	0.40	.28234	.5445
PCSK9	−1.29	0.40	.00876	.2017
CATZ	−1.29	0.40	.04350	.2830
Kininogen_HMW	−1.37	0.38	.08360	.3495
IGFBP_4	−1.37	0.38	.03957	.2800
Cathepsin_B	−1.38	0.38	.00473	.1869
Phosphoglycerate_mutase_1	−1.46	0.36	.0435	.2830
Histone_H2A_z	−1.51	0.34	.00838	.2007
FETUB	−1.52	0.34	.05755	.3169
Clusterin	−1.55	0.34	.00521	.1892
Plasminogen	−1.56	0.33	.00596	.1928
Amyloid_precursor_protein	−1.60	0.32	.0251	.2441
PCI	−1.62	0.32	.01463	.2079
Integrin_aVb5	−1.62	0.32	.00678	.1928
PTHrP	−1.65	0.31	.00122	.1370
CD39	−1.72	0.30	.00039	.1368
MIS	−1.73	0.30	.00130	.1370
PAPP_A	−1.76	0.29	.01554	.2079
Antithrombin_III	−2.31	0.20	.00490	.1869

FDR-BH, Benjamini-Hochberg FDR; *GSVA*, gene set variation analysis.

LTA_4-hydrolase activity within these neutrophilic subjects, which further support a non-T2 phenotype.[49]

We have previously defined patients with asthma according to their sputum molecular phenotypes.[3] The FZ-DOWN signature was enriched in TAC2 patients, which suggests that FZ may be useful for T2-low severe neutrophilic patients with asthma. Pathway analysis of the potential FZ responders versus nonresponders highlighted the importance of neutrophil degranulation products along with signaling downstream of Toll-like receptors, cytokine/chemokines including neutrophil-associated mediators, and chemoattractant receptors such as CXCL10, CXCL11, CXCR1, and CXCR2, suggesting an activated neutrophil phenotype. Although previously defined pathways such as the NLRP3 inflammasome within TAC2 were not specifically enriched in the FZ PR versus PNR subjects, factors associated with inflammasome activation including IL-1α and IL-1RAP are present.[50]

At the cellular level, a significant increase in the percentage of airway neutrophils (75.5% vs 35.6%) and a significant decrease in the percentage of airway macrophages (18.9% vs 32.5%) in the FZ PR group were observed. Macrophages phagocytose apoptotic neutrophils and contribute to inflammation resolution. It is interesting to speculate whether a reduced number of airway macrophages observed could adversely impede neutrophil clearance, thus promoting the elevated levels of airway neutrophils in this endotype of asthma. Defects in neutrophil apoptosis and/or clearance leading to airway neutrophilia have previously been reported in a small cohort of severe atopic patients with asthma with a low-eosinophilic phenotype (≤3% sputum eosinophils).[51]

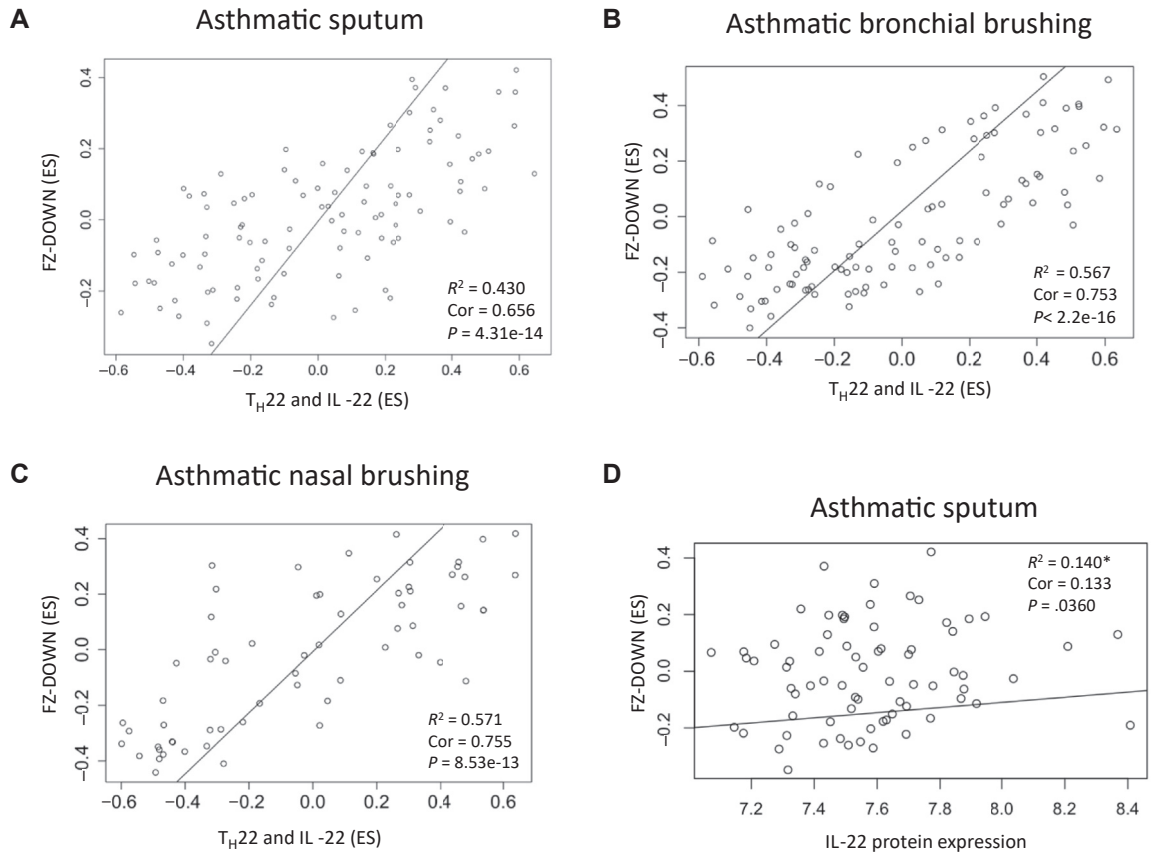

FIG 5. Correlation of the transcriptomic ES of the signature of genes downregulated by FZ treatment (FZ-DOWN) in lesional samples from patients with AD against the ES of the T_H22/IL-22 pathway genes in (**A**) sputum, (**B**) bronchial brushings, and (**C**) nasal brushings of subjects with asthma and against sputum IL-22 protein abundance in the sputum of subjects with asthma (**D**). The correlation for sputum IL-22 protein was controlled for age, sex, and body mass index.

We have previously shown that GM-CSF/CSF2RB- and interferon-activated macrophages and lower enrichment of eosinophils were associated with childhood asthma.[52] The AD disease signature indicates that AD, although generally seen as a T2-dominant disease, also has different degrees of non-T2–driving pathways including T_H1, T_H17, T_H22, and inflammatory pathways.[20] Both GM-CSF and IFN pathways were also enriched within the FZ PR population and interestingly, the PR-PNR pathways also indicated the enrichment of IL-10 signaling, which is involved in the suppression of IL-5 and GM-CSF expression and eosinophil apoptosis.[53] These pathways may also represent therapeutic targets in these patients with SA.

The AD-DOWN signature is not enriched in asthmatic peripheral blood but shows some enrichment in airway samples. This signature includes lipid pathways and pathways associated with dysregulated dermal epithelial function, which indicates that remodeling of epithelial tissues is more prevalent in severe neutrophilic asthma airways. These pathways are also upregulated by FZ, which suggests that FZ may also have an impact on asthmatic airway epithelial cell barrier function.

IL-22 possesses potential proinflammatory and anti-inflammatory roles in asthma.[11,15,16] In mouse models of allergic sensitization and challenge, IL-22 attenuates established T_H2 cell–mediated allergic inflammation *in vivo*.[11,54] However, IL-22 promotes allergic inflammation in similar mouse models at the onset of allergic asthma,[11,18] supporting the view that IL-22 may be involved in the atopic march.[17] Although data from mouse models suggest that anti–IL-22 may be efficacious in early-onset allergic asthma, our analysis would indicate that IL-22 might have a pathogenic role in those with neutrophilic inflammation with lower IgE levels.

In our analysis, IL-22 mRNA expression did not correlate with FZ-response signatures in blood, sputum, nasal, and bronchial brushings, whereas there was a significant correlation with sputum IL-22 protein and with the T_H22/IL-22 gene signature in sputum, bronchial, and nasal brushings. This may reflect the local expression of IL-22 protein in the airways, which is not detected at the mRNA level or is not observed because of lack of proteomics data for bronchial and nasal brushings. However, the upregulation of the FZ-DOWN signature does indicate a significant impact of IL-22 on downstream signaling.

This study has several strengths and also some limitations. We derived a gene signature from skin lesions of subjects with AD (AD-UP) and also from patients with a good clinical response and a clear transcriptomic response to FZ after 12 weeks of treatment (FZ-DOWN) to provide evidence for target engagement in the lesional tissue. We used the large data-rich U-BIOPRED cohort to define subsets of patients who are more likely to respond to FZ

and validated this in a separate cohort of patients with SA. Importantly, we were able to demonstrate markers of high enrichment of this response signature in nasal brushings and peripheral blood. However, we do not have evidence that the changes seen in the lesional skin of patients with AD with FZ also occur in the airways of patients with asthma. Animal models of severe neutrophilic or mixed granulocytic asthma may be used to address this issue. In asthma, baseline levels of IL-22 mRNA did not correlate with FZ-DOWN signature as predicted from the AD data. This suggests that additional mechanisms may be involved in the asthmatic airway compared with the skin. These mechanisms may be linked because there is a strong correlation between the T_H22/IL-22 and FZ-DOWN signatures. The good correlation of both IL-22 sputum protein abundance and T_H22/IL-22 signature ES with the FZ-DOWN signature ES within nasal brushings indicates a potential alternative readily accessible approach for identifying possible responder populations. Although these data were validated in a separate cohort with SA, we have not measured the stability of the FZ-response signature over time and whether this changes with T2-directed biologics.

This novel approach of molecularly characterizing clinical superresponders to an antibody drug in one disease followed by probing other disease databases may be a more effective way of identifying PRs at the endotypes level compared with looking at drug-target levels alone. By exploiting preexisting databases and clinical trial data, this approach could lead to a reduction in drug development time and research costs. The greatest enrichment of the FZ PR signature was observed in severe neutrophilic patients with asthma. Furthermore, we found that blood and sputum gene expression and the expression of several proteins in sputum can predict patients with asthma with a high enrichment of an FZ-response signature in the airway. This stratification process will need validation in a controlled clinical trial, while at the same time examining the long-term efficacy and side-effect profile of FZ in endotypes of SA.

Clinical implications: Identification of transcriptomic drug-response signatures in the target tissue of a chronic immune disease may be used in another disease to stratify subjects for subsequent clinical trials or treatment.

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