Received: 8 June 2023 | Revised: 27 July 2023 | Accepted: 12 August 2023

DOI: 10.1111/all.15869

Allergy EUROPEAN JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY EAACI WILEY

LETTER

Transcriptomics reveals new regulatory mechanisms involved in benralizumab response

To the Editor,

Benralizumab, a monoclonal antibody targeting IL5RA, significantly improves asthma symptoms and quality of life in severe asthmatic patients. However, understanding the factors influencing treatment response remains a challenge. In this prospective observational study, we analyzed transcriptomic changes in peripheral whole blood samples from 15 severe asthmatic patients treated with benralizumab for 6 months. The study included patients with two frequent comorbidities: nasal polyposis (CRSwNP) and NSAID-exacerbated respiratory disease (N-ERD) (Appendix S1 and Table S1).

We analyzed differential gene expression on paired samples, comparing gene expression levels before and after treatment, and identified 216 differentially expressed genes (DEGs) (Figure S1A,B; Appendix S1). Previous studies have shown a reduction in gene expression inherent to the depletion of eosinophil counts upon benralizumab treatment.[1,2] While gene expression generally decreased after treatment, we observed that many differentially expressed genes were not correlated with eosinophil counts (Figure 1A, Figure S1B). In a previous transcriptome analysis of asthmatic patients (Asthma DEG study), we identified 109 DEGs.[3] By comparing both studies, we identified 16 common genes (Table 1†, Figure S2A). Among them, we validated *CYSLTR2*, *IL5RA*, *CLC*, *PTGDR2*, and *SMPD3* as potential asthma biomarkers in our study population (Figure S3).

Furthermore, we identified three DEG clusters after benralizumab treatment (Figure 1A, File S1). Cluster 1 was comprised of downregulated eosinophil-correlated genes. Cluster 2 comprised predominantly upregulated genes, and Cluster 3 consisted mainly of downregulated genes, both uncorrelated to eosinophils. The presence of non-protein coding genes in Clusters 2 and 3 suggested the involvement of additional layers of regulatory processes. Thus, we examined the trans-acting regulatory target RNAs for six differentially expressed long non-coding RNAs (lncRNAs), considering their base pairing with complementary sequences (Table S2, Appendix S1). Such interactions between lncRNAs and RNAs can regulate gene expression, including mRNA stability, localization, or translation.[4] Our findings suggest that specific lncRNAs may contribute to the observed pathway changes during benralizumab treatment, and interestingly, these non-eosinophil-correlated lncRNAs share biological processes with the DEGs such as "Immune system process" and "Regulation of signaling" (File S2). That implies that expression changes in other blood cell types might be masked by eosinophil counts, highlighting the regulatory role of non-coding RNAs and other DEGs and their potential in uncovering the broader transcriptomic effects of benralizumab beyond eosinophil reduction.

CRSwNP and N-ERD are frequent comorbidities in severe asthma, worsening symptoms, and decreasing quality of life.[5] We investigated DEG patterns in asthma patients with and without CRSwNP and N-ERD, identifying 20 shared downregulated genes (Table 1‡, Figure 1B). When comparing these genes with those upregulated in asthma (Asthma DEG Study), we identified a common subset of 10 genes that could define asthma-related inflammation (Figure 1C, Table 1†‡).

A random forest model was used to identify gene expression changes that could predict benralizumab treatment response in severe asthma patients. First, we applied a holistic approach, categorizing the response to benralizumab by considering relevant clinical variables and quality of life measures (Table S3, Appendix S1). Out of the three candidate genes (*FBN1*, *CCR3*, and *SRGAP3*) identified in the transcriptomic study as potential predictors of benralizumab response, *FBN1* could be validated as a potential biomarker for super response (Figure 1D,E, Figure S4). Interestingly, mutations in *FBN1* have been linked to impaired lung function.[6] Further research is needed to explore the clinical implications of *FBN1* in asthma and its potential role in benralizumab response.

These findings provide novel insights into possible regulatory mechanisms underlying treatment response with benralizumab, thus contributing to the development of personalized precision medicine for severe asthma management. Further studies are needed to validate these findings in larger patient cohorts to explore the underlying molecular mechanisms.

FUNDING INFORMATION

This study has been funded by the projects PI20/00268, funded by Instituto de Salud Carlos III (ISCIII) and co-funded by the European Union; RD21/0002/0054 and PMP22/00124, funded by Instituto de Salud Carlos III (ISCIII) and European Union -NextGeneration EU, Mecanismo para la Recuperación y la Resiliencia (MRR); IMP/00009,

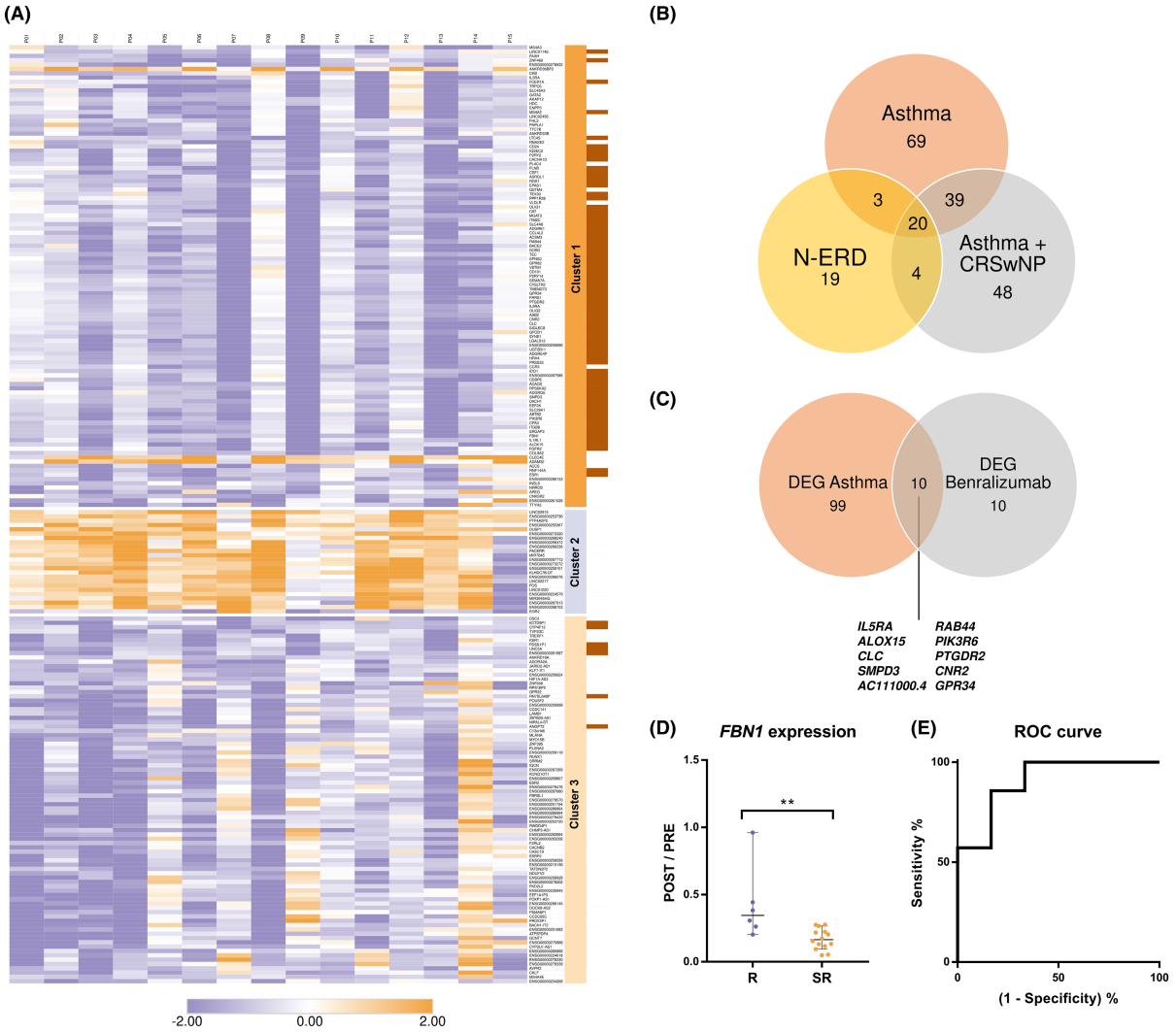

FIGURE 1 (A) Hierarchical clusters of DEGs based on their normalized fold changes from baseline to Month 6 of treatment. The right color bars indicate the three clusters. Brown bars highlight genes correlated with eosinophils. (B) DEGs associated with Asthma ($n=5$), Asthma with CRSwNP ($n=7$), and N-ERD ($n=3$). (C) DEGs common to the three phenotypes and DEGs in asthmatic patients. (D) *FBN1* normalized expression in Responder (R) and Super Responder patients (SR). (E) ROC curve of the predictive super response value of *FBN1* expression based on the probabilities of classifying patients as SR or R according to *FBN1* normalized expression. $^{**}p<0.01$.

funded by Instituto de Salud Carlos III (ISCIII) and European Regional Development Fund "A way to make Europe;" by the Grant PID2021-125117OB-I00 funded by MCIN/AEI/https://doi.org/10.13039/501100011033 and by "ERDF A way of making Europe," by the "European Union;" and by the Grant IES161P20, funded by Consejería de Educación de la Junta de Castilla y León co-funded by FEDER funds.

AUTHOR CONTRIBUTIONS

ID, MI-G, CS, and ME designed the study. ID, MG-M, JR-G, CM-G, and FJM-B recruited patients and collected blood samples and clinical data. JCT performed the bioinformatics analyses for sequencing data. ME, JP-P, JCT, AG-S, and CS performed analyses for data. ME, JP-P, AG-S, and ID performed the analyses for clinical data. ME, JP-P, EM-J, AG-S, MG-G, and NM performed the experimental methodology and validation experiments. ME, CS, ID, MI-G, and JP-P were the major contributors in writing the manuscript. All authors read and approved the final manuscript.

ACKNOWLEDGMENTS

The authors would like to express their gratitude to the staff of the Allergy Department at Salamanca University Hospital, particularly Milagros González Prieto, Cristina Regalado González, and Rosa Aguadero Martín, for their valuable collaboration in providing samples and retrieving clinical information. The authors also thank the Genomic Unit of the Salamanca Cancer Research Center (CSIC-Universidad de Salamanca) for their technical guidance and support, also to Fundación del Instituto de Estudios de Ciencias de la Salud de Castilla y León (IECSCYL) and the Institute of Biomedical Research of Salamanca (IBSAL).

TABLE 1 Differentially expressed genes shared among study groups.

				Asthma DEGs		Benralizumab DEGs	
		Ensembl ID	Gene name	Fold change	pAdj	Fold change	pAdj
†	‡	ENSG00000161905	*ALOX15*	+2.45	3.91E-05	−334.53	3.20E-37
	‡	ENSG00000103355	*PRSS33*			−154.76	1.87E-28
†	‡	ENSG00000250696	*AC111000.4*	+2.01	3.31E-05	−50.42	4.37E-27
	‡	ENSG00000205927	*OLIG2*			−32.06	1.68E-31
	‡	ENSG00000213759	*UGT2B11*			−29.51	4.83E-43
†	‡	ENSG00000091181	*IL5RA*	+2.22	8.63E-08	−25.74	2.52E-41
	‡	ENSG00000115602	*IL1RL1*			−14.89	1.62E-29
†	‡	ENSG00000105205	*CLC*	+2.05	4.63E-06	−14.22	7.44E-31
	‡	ENSG00000131203	*IDO1*			−8.95	1.53E-21
†		ENSG00000268758	*ADGRE4P*	+1.94	2.47E-04	−8.93	8.25E-17
†		ENSG00000134489	*HRH4*	+1.93	7.21E-07	−7.90	8.29E-21
†	‡	ENSG00000183134	*PTGDR2*	+1.99	2.64E-06	−6.44	1.74E-17
†	‡	ENSG00000103056	*SMPD3*	+2.17	1.44E-07	−4.14	1.67E-28
	‡	ENSG00000171657	*GPR82*			−3.91	2.08E-13
†	‡	ENSG00000255587	*RAB44*	+1.74	8.10E-06	−3.83	8.29E-26
	‡	ENSG00000163751	*CPA3*			−3.82	6.97E-20
†	‡	ENSG00000276231	*PIK3R6*	+1.67	9.12E-06	−3.50	1.92E-24
†		ENSG00000152207	*CYSLTR2*	+1.84	1.15E-09	−3.14	2.84E-09
	‡	ENSG00000186204	*CYP4F12*			−2.97	7.14E-20
†		ENSG00000133317	*LGALS12*	+1.58	8.83E-05	−2.89	9.70E-06
†	‡	ENSG00000188822	*CNR2*	+1.53	3.41E-02	−2.82	2.53E-13
	‡	ENSG00000140263	*SORD*			−2.73	2.75E-09
†	‡	ENSG00000171659	*GPR34*	+1.83	1.27E-08	−2.13	1.59E-11
†		ENSG00000174837	*ADGRE1*	+1.65	2.08E-06	−2.11	5.69E-06
	‡	ENSG00000131018	*SYNE1*			−2.09	1.06E-16
†		ENSG00000050438	*SLC4A8*	+1.60	1.23E-04	−1.60	2.58E-04

Note: (†) Genes upregulated in asthma (Asthma DEG study) and downregulated after benralizumab treatment; (‡) DEGs common in the three phenotypes (asthma, asthma with CRSwNP, and NERD) upon benralizumab treatment (benralizumab DEG study).

CONFLICT OF INTEREST STATEMENT

In the last 3 years, Miguel Estravís has received payment for lectures from SANOFI. Asunción García-Sánchez has received payment for lectures from Leti. Jacinto Ramos-González has received payments for lectures from Astra-Zeneca, Chiesi, GSK, Novartis, Sanofi, Menarini, and Boehringer; for a consultancy from Astra-Zeneca, GSK, Novartis, and Sanofi. María Gil-Melcón has received payment for lectures from Astra-Zeneca, GSK, Novartis, and Sanofi. Ignacio Dávila has received payment for lectures, including service on speaker's bureaus from Allergy Therapeutics, Astra-Zeneca, Chiesi, Diater, GSK, Leti, Novartis, and Sanofi; for a consultancy from Allergy Therapeutics, ALK-Abello, Astra-Zeneca, GSK, Merck, MSD, Novartis, and Sanofi; and grants for Thermofisher Diagnostics. The rest of the authors declare no conflict of interest. The funders had no role in the study's design, in the collection, analyses, or interpretation of data, in the writing of the manuscript, or in the decision to publish the results.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Sequence Read Archive (NCBI) at https://www.ncbi.nlm.nih.gov/sra, reference number PRJNA997234.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.