DR MARIA C OVEJERO-BENITO (Orcid ID : 0000-0003-4082-5165)

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Epigenetic biomarkers associated with anti-TNF drugs response in moderate-to- severe psoriasis

Running head: Pharmacoepigenetics of anti-TNF drugs in psoriasis

M.C. Ovejero-Benito[1], T. Cabaleiro[1*], A. Sanz-García[2*], M. Llamas-Velasco[3], M.S. Rodríguez[1], R. Prieto-Pérez[1], M. Talegón[1], M. Román[1], D. Ochoa[1], A. Reolid[3], E. Daudén[3*], F. Abad-Santos[1,4*]

[1]Clinical Pharmacology Department, Hospital Universitario de la Princesa, Instituto Teófilo Hernando, Universidad Autónoma de Madrid (UAM), Instituto de Investigación Sanitaria la Princesa (IIS-IP), Madrid, Spain

[2]Clinical Neurophysiology Department, Hospital Universitario de la Princesa, Instituto de Investigación Sanitaria la Princesa (IIS-IP), Madrid, Spain

[3]Dermatology Department, Hospital Universitario de la Princesa, Instituto de Investigación Sanitaria La Princesa (IIS-IP), Madrid, Spain

[4]Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd), Instituto de Salud Carlos III, Madrid, Spain

*: F Abad-Santos and E Daudén contributed equally to the manuscript

*: T Cabaleiro and A Sanz-García contributed equally to the manuscript.

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Corresponding author:

Francisco Abad-Santos

Clinical Pharmacology Department

Hospital Universitario de la Princesa

Diego de León 62, 28006 Madrid, Spain

Tel: 34 915202425; Fax: 34 915202540

E-mail: francisco.abad@salud.madrid.org

Dear editor

It has recently been discovered that epigenetic modifications, specially DNA methylation, participates in the pathology of psoriasis [1]. DNA methylation is a covalent modification dynamic and heritable that takes place in Cytosine-Phosphate-Guanine (CpG) sites and may exert transcriptional effects [2]. Although anti-tumour necrosis factor α (TNF) therapies (adalimumab, etanercept and infliximab) are efficient drugs for moderate-to-severe psoriasis, around 30–50% of psoriasis patients present an inadequate response [3]. This is the first study that search for epigenetic markers that could predict anti-TNF drugs response.

This evaluation was designed as unicentric, non-interventional, prospective and observational. The protocol and informed consent document complied with Spanish legislation. Effectiveness of anti-TNF agents was evaluated by Psoriasis Area and Severity Index (PASI). Our study included 70 Caucasians patients that presented moderate-to-severe plaque psoriasis and were treated with anti-TNF drugs. Blood samples were taken when the patients attended to the period medical follow-up visits. Excellent responders (ER) and partial insufficiently/non-responders (PR) to anti-TNF drugs were selected to increase the probability of finding biomarkers of drug response as recommended in a

previous genetic analysis [4]. ER achieved a PASI90 response at 3 and 6 months (N=49) while PR did not achieve a PASI75 response (N=21) (Table 1A).

DNA was extracted from peripheral blood samples using the MagNa Pure® System (Roche Applied Science). EZ DNA Methylation Kit (Zymo Research) was used for the bisulphite conversion of 1,000 ng of genomic DNA. Infinium methylation assay was carried out with the Illumina Infinium Human Methylation 450 Bead Chip (Illumina Inc) [5] by Making Genetics (Making Genetics SL) (http://www.making-genetics.eu/es/). Data analysis was performed using the ChAMP pipeline [6] resulting in a dataset of 433,237 CpG sites with good hybridization quality. To reduce possible bias due to cell heterogeneity, DNA methylation data were corrected with the algorithm described by Houseman et al. (2012) [7] and adjusted for covariates gender and age. As no differentially methylated sites (DMSs) were found between smokers and non-smokers, tobacco habit was not included as a covariate.

R statistical software was used to compute a moderated t-test adjusted by the batch effect (the ratio of the methylation value (m-value) to its standard error) for categorical variables. In addition, a linear regression model was used to analyze the potential associations between m-values and continuous variables such PASI at 3 and 6 months, that were performed with limma [8]. Raw p-values were corrected using the Benjamini–Hochberg multiple comparison procedure for false discovery rate. Adjusted p-value lower than 0.05 was considered significant.

No DMSs were found between ER and PR patients to anti-TNF drugs. Furthermore, methylation analysis of adalimumab, etanercept and infliximab were performed independently (Table 1B). No significant DMSs were found between ER and PR patients to infliximab or etanercept treatment. However, three CpGs were hypermethylated in PR patients (N=4) with respect to ER patients to adalimumab (N=21): cg18837178 (located in a non-coding RNA), cg23132469 (*TAS1R2*, Taste 1 Receptor Member 2 gene), cg05221720 (*COL9A1* collagen type IX alpha 1 chain gene) (Table 1C).

A linear regression model showed that there were no association between baseline PASI and PASI at 3 months and m-values of any of the CpG analyzed. Nevertheless, a positive correlation was observed between PASI at 6 months and m-values of cg09141835 thus suggesting that this site is hypermethylated in patients with a poorer response to anti-TNF drugs. cg09141835 is located in the CBFA2T3 gene, which encodes a member of the myeloid translocation gene family that interacts with DNA-bound transcription factors. A negative correlation between PASI at 6 months of treatment and the m-values of the cg23446055 and cg03242666 was also observed, suggesting that these two CpG sites tend to be hypomethylated in patients with a poorer response to anti-TNF drugs. cg23446055 is located in the *PRELID2* gene (the proteins of relevant evolutionary and lymphoid interest (PRELI) domain containing 2), a phospholipid transporter localized in the mitochondria. cg03242666 is located in the 5´ UTR region of the *PMP22* (peripheral myelin protein 22) gene (Table 1D).

This study presents several limitations such as the small sample size. Furthermore, as anti-TNF drugs are prescribed to moderate-to-severe patients resistant to conventional systemic treatment, some of the patients were previously treated with drugs that may affect methylation values such as methotrexate [9]. This is an observational study that did not interfere with the clinical practice. Thus, blood extraction of the patients took place when the patients attended for the consultation during anti-TNF treatment. Therefore, methylation values may be biased by anti-TNF drug intake [10]. Further development of longitudinal studies that analyze methylation differences before and after the treatment with these drugs could help us finding epigenetic biomarkers that could predict anti-TNF drug response in psoriatic patients. Nevertheless, this is the first study searching for epigenetic markers that could predict anti-TNF drugs treatment in moderate-to-severe psoriasis patients.

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A) Clinical and demographic characteristics of the patients that participated in this study.

	Patients (N=70)	Excellent Responders (ER) (N=49)	Partial insufficientely/ Non-Responders (PR) (N=21)	Statistical significance
Age (years)	47.1 ± 14.6	45.4 ± 14.2	51.0 ± 15.0	p=0.137
Age at onset of psoriasis (years)	25.8 ± 11.6	25.4 ± 11.0	26.7 ± 13.1	p=0.679
Men (%)	43 (61.4)	31 (63.3)	12 (57.1)	p=0.789
Weight (kg)	75.5 ± 12.9	75.0 ± 12.7	76.8 ± 13.6	p=0.595
Psoriasis type I [1](%)	61 (87.1)	43 (87.8)	18 (85.7)	p=1.000
Psoriasis type II [2](%)	9 (12.9)	6 (12.2)	3 (14.3)	
Patients with PsA (%)	17 (24.3)	13 (26.5)	4 (19.0)	p=0.561
Age at first biological agent	41.1 ± 13.3	37.9 ± 11.8	47.8 ± 14.1	p=0.010*
Baseline PASI	21.7 ± 11.4	22.0 ± 11.7	21.0 ± 11.1	p=0.748
PASI at 3	3.44	0.61	10.04	p=0.000*

B) EWAS association analysis performed and the results obtained.

Adjusted by gender and age

Variable	Number	Comparison	Total	Hyper	Hypo
Anti-TNF drug global response	70	ER (N=49) vs PR (N=21)	-	-	-
Adalimumab response	25	ER (N=21) vs PR (N=4)	3	3	-
Etanercept response	27	ER (N=16) vs PR (N=11)	-	-	-
Infliximab response	18	ER (N=12) vs PR (N=6)	-	-	-
Baseline PASI	70	LC	-	-	-
PASI at 3 months	70	LC	-	-	-
PASI at 6 months	69	LC	3	1	2

C) Significant correlation between Differentially Methylated Sites in ER (n=21) and PR (n=4) patients to adalimumab

months	± 5.88	± 0.95	± 6.02	
PASI at 6 months	3.69 ± 8.38	0.46 ± 0.79	12.2 ± 12.2	p=0.000*
Adalimumab (%)	25 (35.7)	21 (42.9)	4 (19.0)	p=0.384
Etanercept (%)	27 (38.6)	16 (32.7)	11 (52.4)	
Infliximab (%)	18 (25.7)	12 (24.5)	6 (28.6)	
PT Methotrexate (%)	43 (61.4)	31 (63.3)	12 (57.1)	p=0.630
PT Ciclosporine (%)	28 (40.0)	19 (38.8)	9 (42.9)	p=0.749
PT Acitretin (%)	46 (65.7)	34 (69.4)	12 (57.1)	p=0.323
PT Phototherapy (%)	33 (47.1)	24 (49.0)	9 (42.9)	p=0.638
PT Efalizumab (%)	56 (80.0)	40 (81.6)	16 (76.2)	p=0.602

CpG site	**CHR**	**Gene Name**	**CpG-site neighborhood**	**adj. p-value**
cg18837178	5	*NA*	N_Shelf	0.003
cg23132469	1	*TAS1R2*	Island	0.014
cg05221720	6	*COL9A1*	N_Shore	0.049

D) Significant correlation between PASI at 6 months and DNA methylation values

CpG site	**CHR**	**Gene Name**	**CpG-site neighborhood**	**adj. p-value**
cg09141835	5	*CBFA2T3*	NA	0.001
cg23446055	16	*PRELID2*	NA	0.002
cg03242666	17	*PMP22*	S_Shelf	0.026

Table 1. A) Characteristics of the patients included is the study. Clinical Data are shown as mean ± SD or number. Statistical differences were analyzed between excellent responders and non-responder patients. T-test and χ^2 were performed for categorical and continuous variables respectively. B) Summary of the EWAS association analysis performed and the results obtained for the different variables analyzed. C) Significant correlation between Differentially Methylated Sites in ER and PR patients to adalimumab D) Significant correlation between Differential Methylated Sites in peripheral blood samples and specific DNA methylation values with respect to PASI at 6 months. Only significant DMSs are shown (adj. p-value <0.05).

Abbreviations [1]: early-onset psoriasis (<40 years); [2]: late-onset psoriasis (≥40 years); PsA: psoriatic arthritis; PASI: Psoriasis Area and Severity Index; SD: standard deviation; PT: Patients previously treated with (the following drug). Gene: gene name according to the USC Gene Nomenclature Committee. CHR: chromosomal position of the CpG according to National Center for Biotechnology Information (NCBI). CpG-site

neighborhood: location of the gene-associated CpG-site(s) within the CpG-site neighborhood. adj. p-value: Moderate t-test p-values adjusted for false discovery rate correction. DMSs: Differentially methylated sites, LC: linear correlation of the m-value of every CpG with a continuous variables. Hypo: hypomethylated, Hyper: hypermethylated. *NA*: Non available; *TAS1R2*: taste 1 receptor member 2; *COL9A1*: collagen type IX alpha 1 chain gene. *CBFAT3*: Core-Binding Factor, Runt Domain, Alpha Subunit 2; Translocated To, 3; *PRELID2*: proteins of relevant evolutionary and lymphoid interest (PRELI) domain 2; *PMP22*: Peripheral Myelin Protein 22.

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Conflicts of interests: F Abad-Santos and D Ochoa have been a consultant or investigator in clinical trials sponsored by the following pharmaceutical companies: Abbott, Alter, Chemo, Farmalíder, Ferrer, Galenicum, GlaxoSmithKline, Gilead, Janssen-Cilag, Kern, Normon, Novartis, Servier, Teva and Zambon. E Daudén has potential conflicts of interest (advisory board member, consultant, grants, research support, participation in clinical trials, honoraria for speaking, and research support) with the following pharmaceutical companies: AbbVie (Abbott), Amgen, Janssen-Cilag, Leo Pharma, Novartis, Pfizer, MSD, and Celgene. M. Llamas Velasco has potential conflicts of interest as she has participated in clinical trials with Abbvie (Abbott), Janssen-Cilag, Leo Pharma, Pfizer and Celgene. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.