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Histone modifications associated with biological drug response in moderate-to-severe psoriasis

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Abstract

Introduction: Epigenetic factors play an important role in psoriasis onset and development. Biological drugs are used to treat moderate-to-severe psoriasis patients resistant to conventional systemic drugs. Although they are safe and effective, some patients do not respond to them. Therefore, it is necessary to find biomarkers that could predict response to these therapies.

Objective: To find epigenetic biomarkers that could predict response to biological drugs (ustekinumab, secukinumab, adalimumab, ixekizumab).

Materials and methods: Peripheral blood mononuclear cells (PBMCs) were isolated from 39 psoriasis patients treated with biological therapies before and after drug administration and from 42 healthy subjects. Afterwards, histones were extracted from PBMCs. Four histone modifications (H3 and H4 acetylation, H3K4 and H3K27 methylation) were determined by ELISA. Data were analysed by IBM-SPSS v.23.

Results and conclusions: Psoriasis patients presented reduced levels of acetylated H3 and H4 and increased levels of methylated H3K4 compared to controls. Non-significant changes were observed after treatment administration in any of the histone modifications analysed. Nevertheless, significant changes in methylated H3K27 were found between responders and non-responders to biological drugs at 3 months. As 28% of these patients also presented psoriatic arthritis (PsA), the former analysis was repeated in the subsets of patients with or without PsA. In patients without PsA, significant changes in methylated H3K4 were found between responders and non-responders to biological drugs at 3 and 6 months. Although further studies should confirm these results, these findings suggest that H3K27 and H3K4 methylation may contribute to patients' response to biological drugs in psoriasis.

Abbreviations: µg, microgram/s; µl, microlitres; BMI, body mass index; ChIP seq, Chromatin immunoprecipitation sequencing; DMSO, dimethyl sulfoxide; DTT, DL-dithiothreitol; ELISA, enzyme-linked immunosorbent assay; H2A, histone 2A; H2B, histone 2B; H3, histone 3; H3K27, histone 3 modified in lysine 27; H3K36, histone 3 modified in lysine 36; H3K4, histone 3 modified in lysine 4; H3K79, histone 3 modified in lysine 79; H3K9, histone 3 modified in lysine 9; H4, histone 4; H4K20, histone 4 modified in lysine 20; IBM, International Business Machines Corporation; IL-12/23/17, interleukin 12, 23 or 17; IQR, inter-quartile range; min, minute/s; ml, millilitres; nm, nanometres; PASI75, a reduction of 75% from the baseline PASI; PASI90, a reduction of 90% from the baseline PASI; PASI, Psoriasis Area Severity Index; PBMCs, peripheral blood mononuclear cells; PBS, phosphate-buffered saline; PsA, psoriatic arthritis; RPMI medium, Roswell Park Memorial Institute medium; rpm, revolutions per minute; SD, standard deviation; SPSS, Statistical Package for the Social Sciences; TNF, tumor necrosis factor; WBC, white blood count.

Ovejero-Benito and Reolid, contributed equally to the manuscript. Daudén and Abad-Santos contributed equally to the manuscript.

KEYWORDS
biological drugs, biomarkers, epigenetics, pharmacoepigenetics, psoriatic arthritis

1 | INTRODUCTION

Psoriasis is a chronic and immune-mediated inflammatory skin disorder that affects 2%-3% of the world population.[1,2] Although psoriasis aetiology is not completely understood, epigenetic changes such as DNA methylation and histone modifications, are involved in this disease.[3–9] Epigenetics is the field that studies the heritable changes in gene expression occurring in the absence of DNA sequence alterations that can be caused by environmental factors.[10,11] DNA is compacted within the nucleus as the result of the complex interaction with histones, generating a structure denominated named chromatin. The basic unit of chromatin is the nucleosome, which is composed by an octamer of histones (two subunits of each of the following histones: histone 2A (H2A), histone 2B (H2B), histone 3 (H3) and histone 4 (H4)) tightly bound to DNA by electrostatic interactions.[12–14] Histone tails can be subjected to post-translational modifications (acetylation, methylation, phosphorylation, ubiquitination, among others) that induce changes in chromatin structure.[12,15] Different histone modifications can occur simultaneously, giving rise to crosstalk between the different marks; thus, a single mark does not determine an outcome by itself.[16] Therefore, there is a histone modification code that determines chromatin function in the nucleus according to the chromatin packaging state and, therefore, gene activity in specific chromatin regions.[10,17] Roughly, euchromatin (the active form of chromatin) is characterized by high levels of acetylation and histone H3 methylated in lysine 4, 36 and 79 residues (H3K4, H3K36 and H3K79). On the other hand, heterochromatin (the inactive form of chromatin) is characterized by low levels of acetylation and high levels of histone H3 methylated in lysine 9 and 27 residues (H3K9, H3K27) and histone H4 methylated in lysine 20 residue (H4K20).[18,19]

There are different approaches to treat moderate-to-severe psoriasis patients resistant to conventional systemic therapies (cyclosporine, acitretin, methotrexate).[20–22] The first biological approaches to treat the disease are anti-TNF drugs such as adalimumab, infliximab and etanercept.[23] Furthermore, alternative biological drugs are used to treat this disease such as ustekinumab (a monoclonal antibody that binds to the p40 subunit shared by IL-12 and IL-23)[24] or secukinumab and ixekizumab (monoclonal antibodies that target IL-17).[25–27] As biological drugs are expensive and, although rarely, they can cause severe secondary adverse effects,[22,28,29] it is necessary to discover non-invasive biomarkers that could predict drug response and facilitate treatment individualization for psoriasis patients.

Epigenetic modifications can explain inter-individual differences in the response to therapy.[30,31] A previous publication from our laboratory was the first to apply pharmacoepigenetics in psoriasis, analysing the associations between DNA methylation and biological drugs response.[32] However, to our knowledge, the changes in histone modifications associated with biological drugs response have not been analysed so far in psoriasis patients.

2 | MATERIALS AND METHODS

2.1 | Study population

This study included 39 psoriasis patients treated with biological drugs from the Dermatology Department of the "Hospital Universitario de La Princesa" in Madrid, Spain. Patients received biological drugs (adalimumab, ixekizumab, secukinumab and ustekinumab) according to drug label. Inclusion criteria were the following: Caucasian patients older than 18 years with plaque psoriasis and evidence of moderate-to-severe disease according to the Consensus Document of the Psoriasis Working Group of the Spanish Academy of Dermatology and Venereology,[33] not treated with conventional systemic therapy or phototherapy in the 30 days previous to the study and who signed the informed consent. Exclusion criteria were the following: subjects having other types of psoriasis (guttate or erythrodermic psoriasis) as the dominant form of the disease, and pregnant women.

Psoriasis Area Severity Index (PASI) was used as effectiveness criteria to evaluate biological drug response at 3-6 months of treatment. Patients that reached at least a reduction of 75% from the baseline PASI (PASI75) were considered as responders to biological drugs. Furthermore, PASI90 (a reduction of 90% from the baseline PASI) was also measured at 3 and 6 months of treatment.

Additionally, this study included 42 healthy controls who participated in different bioequivalence clinical trials from the Clinical Trials Unit of the Clinical Pharmacology Department of the "Hospital Universitario de La Princesa in Madrid," Spain. Inclusion criteria were the following: healthy subjects not taking any medication or herbal products, 18-55 years old, with a body mass index 18.5-30 kg/m^2, who signed the informed consent and agreed to fulfil the requirements of the study. Exclusion criteria were the following: controls suffering from psoriasis or having a family member with psoriasis, the presence of systemic or psychiatric diseases, smoking, lactose allergy, vegetarianism and pregnancy.

The protocols and the informed consent documents complied with Spanish legislation on biomedical and clinical research and were approved by the Research Ethics Committee of "Hospital Universitario de La Princesa."

2.2 | Experimental design

This evaluation was designed as a unicentric, non-interventional, cohort, longitudinal and observational study for moderate-to-severe plaque psoriasis patients. In patients, blood extractions were

performed before (baseline) and after treatment initiation (final), coincident with another blood test required for patients' follow-up (3 or 6 months after the beginning of treatment). In the case of healthy controls, only one blood extraction was performed before the administration of any medication involved in the clinical trials. Nine millilitres (mL) of peripheral blood were extracted from psoriasis patients and healthy controls and collected in a heparin-lithium tube.

2.3 | Enrichment of peripheral blood mononuclear cells from blood samples

In order to minimize the impact of cell heterogeneity in our analysis of blood, peripheral blood mononuclear cells (PBMCs) were isolated by a density gradient centrifugation. Initially, 9 mL of peripheral blood were mixed with 9 mL of Phosphate-buffered saline (PBS, Sigma Aldrich, San Luis, Misuri, USA). This mix was carefully added to 9 mL of Lymphocyte Separation Medium (Cultek) and centrifuged at 92g for 35 minutes using a slow acceleration and deceleration programme. The cellular interface was washed with PBS at 43g for 15 minutes. Cells were re-suspended in Roswell Park Memorial Institute medium (RPMI-1640, Sigma) at 4°C. A 60 μL aliquot was used to measure the White Blood Count (WBC) in an automatic cell counter (MEK-6318K, Celltac, Nihon Kohden Tokyo, Japan). Later, cells were centrifuged and re-suspended in freezing medium (Foetal Bovine Serum containing 10% dimethyl sulfoxide (DMSO) and 1% antibiotic/antimicotic (Sigma)). PBMC extracts were stored at −80°C.

2.4 | Total histone extraction and quantification

Total histones were extracted from PBMCs using EpiQuik™ Total Histone Extraction Kit (Epigentek Group Inc. Farmingdale, NY, USA) following the manufacturer's protocol. Briefly, cells were thawed on ice, washed with PBS and centrifuged at 100g for 5 minutes. After incubating the cells with 1× Pre-lysis Buffer and lysing on ice for 10 minutes, cells were centrifuged at 900g for 5 minutes at 4°C. Then, they were re-suspended in 200 μL of Lysis Buffer, incubated on ice for 30 minutes and centrifuged at 1.380g for 5 minutes at 4°C. Supernatant was mixed with 60 μL of Balance Solution with DL-Dithiothreitol (DTT) immediately. Protein concentration was measured by triplicate using a NanoDrop® ND-1000 Spectrophotometer (Wilmington). Protein extracts were aliquoted and stored at −80°C.

2.5 | Histone modifications analysis

2.5.1 | Global histone H3/H4 acetylation measurement

Histone H3/H4 acetylation was measured with EpiQuik™ Global Histone H3 Acetylation Detection Fast Kit or EpiQuik™ Global Histone H4 Acetylation Detection Fast Kit (Epigentek Group Inc.) respectively, according to the manufacturer's protocol as described previously.[8] Briefly, 1 microgram (μg) of histones diluted in Antibody Buffer were added to wells coated with anti-acetyl histone H3/H4 antibody and detected with a labelled detection antibody followed by a colour development reagent. Absorbance was measured at 450 nm on a microplate reader (Multiskan FC, Thermo Scientific) and the washing steps were performed with a microplate washer (Wellwash™, Thermo Scientific Waltham, Massachussetts, USA). Acetylated H3/H4 histone percentage was calculated according to the manufacturer's protocol. Samples, standards and blanks were analysed in triplicate.

2.5.2 | Global histone H3K4/H3K27 methylation measurement

Histone H3K4/H3K27 methylation was measured with EpiQuik™ Global Histone H3K4 Methylation Assay Kit or EpiQuik™ Global Histone H3K27 Methylation Assay Kit (Epigentek Group Inc.), respectively, according to the manufacturer's protocol. Briefly, 1 μg of histones diluted in Antibody Buffer were added to wells. The methylated histone H3K4/H3K27 was recognized with a high-affinity antibody. The ratio or amount of methylated H3K4/H3K27 can be quantified through horseradish peroxidase (HRP) conjugated secondary antibody. Absorbance was measured at 450 nm on a microplate reader (Multiskan FC, Thermo Scientific). Methylated H3K4/H3K27 proteins were calculated according to the manufacturer's protocol. Samples, standards and blanks were analysed in triplicate.

2.6 | Statistical analysis

Statistical differences in clinical and dermatological variables between responder and non-responder patients were presented as mean ± standard deviation (SD). *T* and chi-squared tests were performed for continuous and categorical variables, respectively.

The percentage of the analysed histone modifications was calculated according to the manufacturer's protocol and was presented as median followed by the inter-quartile range (IQR) (25th and 75th percentiles). Non-parametric tests were used for the analysis of histone modifications: Wilcoxon signed-rank test was applied for within-patients differences (before and after biological drugs treatment) while Mann-Whitney *U* test was used for the comparisons of healthy controls and psoriasis patients and responder and non-responder patients.

Samples obtained from every patient (before and after drug administration) were analysed in the same ELISA plate. Patients and healthy controls were distributed in seven different ELISA plates for every modification tested (Histone H3/H4 acetylation and H3K4/H3K27 methylation). One plate from each analysis was excluded due to the following criteria: the calibration curve showed an *R* value lower than 0.75 or the percentage of outliers were higher than 30%. Thus, the sample size was reduced in all analysed histone modifications. Nevertheless, a previous sensibility study including the results of the seven experiments was performed, obtaining similar results.

Data were analysed by Microsoft Excel (Microsoft Corporation, Alburquerque, Nueva Mexico, USA) and SPSS v23.0 (IBM-SPSS Inc) software. Statistical significance was set at $P \leq 0.05$.

TABLE 1 Summary of demographic and clinical characteristics of the patients that participated in this study

	Psoriasis patients N = 39	Responder patients N = 33	Non-responder patients N = 6	Statistical significance *P*
Age at onset of psoriasis (y)	28.8 ± 13.8	29.0 ± 13.7	27.7 ± 15.7	0.831
Age	46.4 ± 14.1	45.8 ± 13.9	49.5 ± 16.1	0.564
Men (%)	29 (74.4%)	23 (69.7%)	6 (100.0%)	0.308
Psoriasis type I[a] (%)	33 (84.6%)	27 (81.8%)	6 (100.0%)	0.564
Psoriasis type II[b] (%)	6 (15.4%)	6 (18.2%)	0 (0.0%)	
Patients with PsA (%)	11 (28.2%)	10 (30.3%)	1 (16.7%)	0.655
Baseline PASI	14.7 ± 5.6	15.1 ± 5.9	12.7 ± 3.9	0.367
Absolute PASI at 3 months	1.6 ± 2.1	0.8 ± 1.0	5.8 ± 2.1	0.002*
Absolute PASI at 6 months	1.3 ± 2.0	1.0 ± 1.6	3.3 ± 3.2	0.170
Ustekinumab (%)	12 (30.8%)	10 (30.3%)	2 (33.3%)	0.960
Secukinumab (%)	21 (53.8%)	18 (54.6%)	3 (50.0%)	
Ixekizumab (%)	1 (2.6%)	1 (3.0%)	0 (0.0%)	
Adalimumab (%)	5 (12.8%)	4 (12.1%)	1 (16.7%)	

Data are shown as mean ± SD or number (%).
PsA: psoriatic arthritis; PASI: Psoriasis Area and Severity Index; Statistical differences were analysed between responder and non-responder patients. *T* test and chi-squared test were performed for continuous and categorical variables, respectively.
[a]Early-onset psoriasis (<40 y).
[b]Late-onset psoriasis (>40 y).
*$P < 0.05$.

3 | RESULTS

3.1 | Population

The study included 39 moderate-to-severe plaque psoriasis patients treated with biological drugs (29 men and 10 women) and 42 healthy controls (23 men and 19 women). Healthy controls were younger than psoriasis patients (27.1 ± 7.6 vs 46.4 ± 14.1, respectively, $P = 0.000$). The clinical characteristics of psoriasis patients are shown in Table 1. PASI75 at 3 months of treatment was selected as a cut-off point to assess biological drug response. As expected, significant differences were found in PASI at 3 months between responders and non-responders (Table 1). A 28.2% of the psoriasis patients also suffered from psoriatic arthritis (PsA) (n = 11, 7 men and 4 women).

3.2 | Differential histone modification analysis in psoriasis

In order to analyse the histone modifications associated with psoriasis, global H3 and H4 acetylation and H3K4 and H3K27 methylation were analysed in healthy controls and psoriasis patients before treatment initiation (Figure 1). Global histone H3 acetylation was significantly lower ($P = 0.012$) in psoriatic patients (1.17 (IQR: 0.29-2.05), n = 39) than in healthy controls (1.84 (IQR: 1.28-2.64), n = 31; Figure 1A). Furthermore, global histone H4 acetylation was significantly reduced ($P = 0.000$) in psoriatic patients (1.54 (IQR: 0.36-5.02), n = 37) compared to controls (9.88 (IQR: 4.03-15.96), n = 36; Figure 1B). Moreover, the percentage of H3K4 methylation was significantly increased ($P = 0.004$) in psoriatic patients (4.74 (IQR: 1.48-6.93), n = 33) with respect to healthy controls (2.25 (IQR: 0.81-3.59), n = 42; Figure 1C). On the contrary, there were no significant differences in the H3K27 methylation levels between psoriatic patients (1.85 (IQR: 1.21-3.22), n = 33) and healthy controls (2.88 (IQR: 1.63-3.35), n = 40; Figure 1D). In summary, there were differential changes in H3 and H4 acetylation and H3K4 methylation in moderate-to-severe plaque psoriasis patients compared to healthy controls.

In order to determine whether the histone modification patterns were affected by the presence of PsA, the former analyses were repeated with the subsets of patients with and without PsA. Global histone H3 acetylation was significantly lower ($P = 0.008$) in patients without PsA (1.11 (IQR: 0.19-2.00), n = 28) than in healthy controls (1.84 (IQR: 1.28-2.64), n = 31; Figure S1A). In contrast, global histone H3 acetylation showed a non-significant decrease ($P = 0.271$) in PsA patients (1.47 (IQR: 0.97-2.50), n = 11) compared to healthy controls (Figure S1B). Furthermore, global histone H4 acetylation was significantly reduced ($P = 0.005$ and $P = 0.001$) both in patients with PsA (1.61 (IQR: 0.29-6.44), n = 11) or without PsA (1.44 (IQR: 0.37-4.23), n = 26), with respect to healthy controls (9.88 (IQR: 4.03-15.96), n = 36). Besides, the percentage of H3K4 methylation was significantly increased ($P = 0.000$) in patients with PsA (4.91 (IQR: 4.04-6.94), n = 9) with respect to healthy controls (2.25 (IQR: 0.81-3.59), n = 42). However, this increment was not significant ($P = 0.094$) for H3K4 methylation in the patients without PsA (4.05 (IQR: 0.99-6.90), n = 24; Figure S1E,F). On the contrary, there were no significant differences ($P = 0.544$ and $P = 0.393$) in the H3K27 methylation levels between patients with PsA (2.55 (IQR: 1.49-3.02), n = 10)

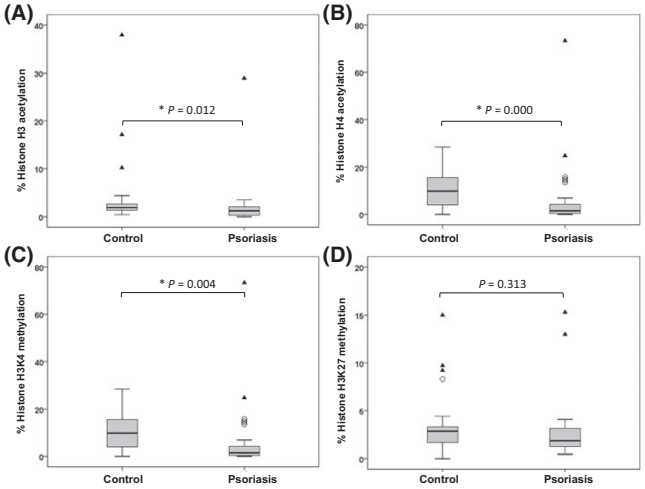

FIGURE 1 Boxplot representation of the global histone acetylation/methylation levels in PBMCs from psoriatic patients and healthy controls. A, Global histone H3 acetylation boxplot of psoriatic patients (n = 39) and healthy controls (n = 31). B, Global histone H4 acetylation boxplot of psoriatic patients (n = 37) and healthy controls (n = 36). C, Histone H3K4 methylation boxplot of psoriatic patients (n = 33) and healthy controls (n = 42). D, Histone H3K27 methylation boxplot of psoriatic patients (n = 33) and healthy controls (n = 40). The bottom of the box represents the 25th percentile while the top of the box represents the 75th percentile. Whiskers extend to more extreme values (1.5 times the inter-quartile range from the box). Circles represent outliers that do not fall in the whiskers while triangles represent extreme outliers (three times the inter-quartile range from the box) (*P < 0.050)

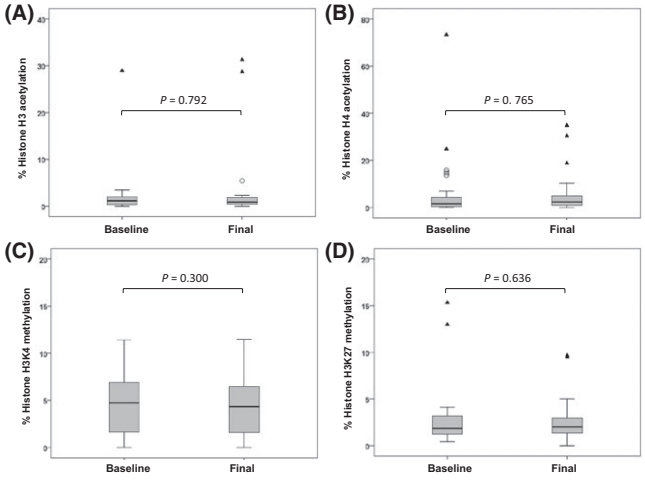

FIGURE 2 Boxplot representation of histone modifications before (baseline) and after treatment with biological drugs (final) in psoriatic patients. A, Global histone H3 acetylation boxplot (n = 39). B, Global histone H4 acetylation boxplot (n = 37). C, Histone H3K4 methylation boxplot (n = 33). D, Histone H3K27 methylation boxplot (n = 33). The bottom of the box represents the 25th percentile while the top of the box represents the 75th percentile. Whiskers extend to more extreme values (1.5 times the inter-quartile range from the box). Circles represent outliers that do not fall in the whiskers while triangles represent extreme outliers (three times the inter-quartile range from the box)

or without PsA (1.58 (IQR: 0.94-3.51), n = 22) and healthy controls (2.88 (IQR: 1.63-3.35), n = 40; Figure S1G,H). In summary, there were differential changes in H3 and H4 acetylation in patients without PsA with respect to controls. Additionally, significant differences were found in H4 acetylation and H3K4 methylation in patients with PsA with respect to healthy controls. Nevertheless, no differences were observed between patients with and without PsA in any of the histone modifications analysed (Figure S2).

3.3 | Analysis of histone modifications associated with biological drugs response

In order to determine whether biological therapies affect histone modifications, their percentages were compared before and after drug administration (Figure 2). No significant differences were found in any of the histone modifications analysed, global H3 acetylation (Figure 2A), global H4 acetylation (Figure 2B), H3K4 methylation (Figure 2C) and H3K27 methylation (Figure 2D).

Equivalent results were found when these comparisons were performed in the subsets of patients with or without PsA (n = 28 and n = 11, respectively) (Figure S3).

Furthermore, the potential association of histone modifications with patient drug response was analysed. Moderate-to-severe plaque psoriasis patients were classified as responders if they achieved a 75% reduction of baseline PASI or non-responders (non-sufficiently responders) if they did not meet this condition. The modified histone levels after drug administration were subtracted from baseline levels. Afterwards, this increment was compared between responder and non-responder patients. Significant differences were not observed either in global histone H3 and H4 acetylation or in H3K4 methylation (Figure 3A-C, respectively) at 3 months of treatment. However, significant differences (P = 0.018) in H3K27 methylation levels were observed between responder (0.51 (IQR: −0.67 to 1.35), n = 28) and non-responder patients (−2.21 (IQR: −3.03 to [−0.02]), n = 5; Figure 3D) at 3 months. Thus, H3K27 methylation levels could potentially discriminate responder and non-responder patients to biological drugs after 3 months of treatment (Figure 3D).

The same analysis was repeated in patients without PsA (Figure S4). Significant differences were not observed either in global H3 and H4 acetylation or in H3K27 methylation (Figures S4A,B,D) at 3 months of treatment. Surprisingly, significant differences were observed in H3K4 methylation (P = 0.044) between responder (−0.44 (IQR: −1.10 to [−0.06]), n = 20) and non-responder patients (0.40 (IQR: 0.10-0.81), n = 4; Figure S4C) at 3 months. Similar results in H3K4 methylation (P = 0.025) between responder (−0.46 (IQR: −1.15 to [−0.05]), n = 19) and non-responder patients (0.56 (IQR: −0.08 to 1.74), n = 5) were observed at 6 months (Figure S5C). The reduced number of patients with PsA (n = 11), especially in the non-responder group (PASI75, n = 1) were insufficient to perform these analyses in this subset at 3 or 6 months of treatment.

Nonetheless, significant differences were not observed between responders and non-responders at 6 months in any of the histone

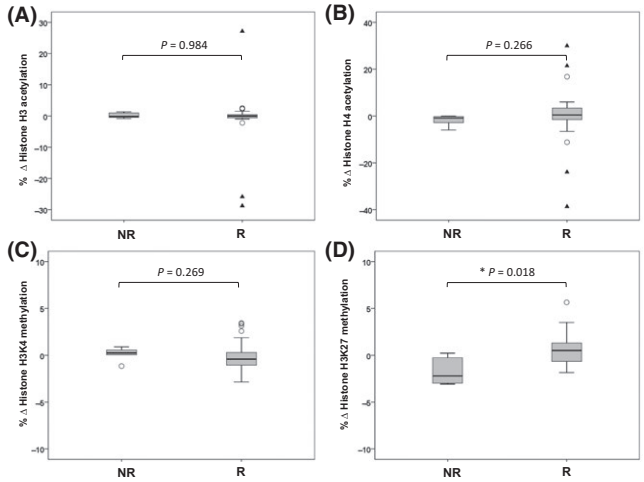

FIGURE 3 Boxplot representation of the association of histone modifications with drug response. A, Comparison of the increments in global histone H3 acetylation after drugs treatment between responders (R, n = 33) and non-responders (NR, n = 6) to biological drugs. B, Comparison of the increments in global histone H4 acetylation after drug treatment between responders (R, n = 32) and non-responders (NR, n = 5) to biological drugs. C, Comparison of the increments in histone H3K4 methylation after drug treatment between responders (R, n = 28) and non-responders (NR, n = 5) to biological drugs. D, Comparison of the increments in histone H3K27 methylation after drug treatment between responders (R, n = 28) and non-responders (NR, n = 5) to biological drugs. Data are shown as median followed by inter-quartile range (25th and 75th percentiles). The bottom of the box represents the 25th percentile while the top of the box represents the 75th percentile. Whiskers extend to more extreme values (1.5 times the inter-quartile range from the box). Circles represent outliers that do not fall in the whiskers while triangles represent extreme outliers (three times the inter-quartile range from the box) (*$P < 0.050$) Δ: Increment in histone modification between before and after treatment administration

modification tested when all the patients were jointly analysed (Figure S6).

Moreover, the association of histone modifications and the achievement of PASI90 at 3 and 6 months were evaluated (Figures S7 and S8, respectively). Nevertheless, no significant differences were observed in any of the histone modifications analysed. These results were confirmed in patients without PsA at 3 and 6 months of treatment (Figures S9 and S10). Moreover, no significant differences were found in patients with PsA at 3 months of treatment (Figure S8). However, these comparisons could not be performed in the subset of patients with PsA at 6 months due to the small number of patients that did not reach PASI90 at 6 months (n = 1).

4 | DISCUSSION

Pharmacoepigenetics is a cutting-edge research field focused on the investigation of epigenetic biomarkers of drug response.[30,31] It has been widely applied for searching biomarkers of drug response and drug resistance in cancer chemotherapy[34–36] or in the prediction of antidepressant response.[37] Furthermore, these studies have focused on the association of DNA methylation with drug response.[34–36] Our laboratory has published the first paper on the pharmacoepigenetics of psoriasis analysing the DNA methylation marks that could predict the response to anti-TNF drugs in psoriasis.[32] Nevertheless, to our knowledge, no study has analysed the histone modifications associated with drug response in psoriasis.

In the first place, the association of histone modifications with psoriasis was tested in PBMCs extracted from healthy controls and patients. Psoriasis patients exhibited significantly reduced levels of acetylated histone H3 and H4 and increased levels of H3K4 methylation with respect to healthy controls. These results are consistent with a previous publication conducted in Chinese population, which showed a significant decrease in H4 acetylation in psoriasis patients with respect to controls.[8] However, this publication failed to find differences between these groups in H3 acetylation and H3K4 methylation levels.[8] The discrepancies found in both studies may be partially explained by the different ethnicity of the study population as this characteristic can affect epigenetics.[38,39] Moreover, healthy controls included in the present study were younger (27.1 ± 7.6) than the controls (36.5 ± 11.7) in the study published by Zhang and collaborators.[8] In conclusion, these results suggest that psoriasis patients present lower levels of acetylation in H3 and H4 and higher levels of methylated H3K4 than controls. In addition, as 28% of the patients included in this study also presented PsA,[40] we wonder whether this condition could alter histone modification patterns. Recent studies have demonstrated that epigenetics play a role in PsA.[41,42] However, to our knowledge, the histone modifications associated with PsA have not been studied so far. Thus, we have analysed the differences in histone modifications between controls and patients with or without PsA. Significant differences in H3 acetylation were observed in patients without PsA with respect to controls. This difference could not be observed in the subset of patients with PsA. On the other hand, H4 acetylation and H3K27 methylation patterns are similar in both patients with and without PsA. In contrast, PsA patients show an increase in H3K4 methylation levels with respect to controls. This increment is not significant between patients without PsA and controls. These results suggest a different participation of histones in both diseases. Nonetheless, no differences were observed between patients with or without PsA in any of the histone modification analysed.

No differences were observed in the baseline histone modification levels between patients suffering from PsA (n = 11) and psoriasis without articular affectation (n = 28). These results could be explained by a lack of effect of PsA as a confounding factor. Nevertheless, as the number of PsA patients is very limited (n = 11), the statistical power may be insufficient to detect differences between those groups.

The decrease in the H3 and H4 acetylation levels has been mostly associated with heterochromatin while the increase in

methylated H3K4 levels is usually associated with euchromatin.[18,19] Nevertheless, the crosstalk between the different histone marks is complex and its transcriptional effect has not been fully clarified.[13,43,44] Thus, further analysis with complementary techniques, such as chromatin immunoprecipitation sequencing (ChIP seq) will be required to determine the specific genes regulated by these modifications in psoriasis and PsA patients.[45]

Moreover, the changes in histone modifications associated with systemic psoriasis therapies were analysed. No significant differences in any of the histone modifications tested were found when their levels were compared before and after biological drugs treatment. Similar results were found when these analyses were carried out within the group of patients with and without PsA.

There are different factors which could not be controlled in this study that may induce epigenetic changes capable of partially masking biological treatment induced histone modifications. Several of these putative confounding factors are diet, hormones, smoking,[46,47] or the different time points of sample collection (at 3 or 6 months). Besides, the inclusion of patients treated with several drugs with different mechanism of action (adalimumab, ixekizumab secukinumab or ustekinumab) could act as a confounding factor. Moreover, as these drugs are highly effective,[25,26] the number of patients that do not respond is low, thus reducing the statistical power of several comparisons. Further studies including a higher number of subjects treated with each drug should be performed in order to confirm these results.

In order to analyse whether there was an association between biological drugs response and histone modifications, the increments in percentages of histone modification after drug administration were compared between responders and non-responders. Non-significant differences in histone H3 and H4 acetylation or histone H3K4 methylation were found between responder and non-responder patients to biological drugs. Nevertheless, non-responders (n = 5) showed a significant decrease in the levels of histone H3K27 methylation compared to responder patients (n = 28) at 3 months of treatment. These results suggest that histone H3K27 methylation may be involved in patient response to biological drugs in psoriasis at 3 months of treatment. In contrast, no association was found between histone modifications and biological drug response at 6 months. When PASI90 (a reduction of 90% from the baseline PASI) was used as a cut-off point of disease improvement instead of PASI75, no association was found between biological drug response and histone modifications at 3 and 6 months. Therefore, these results should be confirmed in large-scale studies.

It has been reported that heterochromatin shows high levels of methylated H3K27.[13,18,19,43,44] Thus, the reduced levels of H3K27 in non-responder patients may be associated with euchromatin and a higher expression of certain genes. Therefore, gene expression imbalance could play a role in the response to biological drugs. Further research performed with complementary techniques such as ChIP seq[45] should be carried out in order to detect the specific genes regulated by these modifications in non-responder patients.

The main limitation of this study is the reduced sample size which is insufficient to perform drug-specific independent analyses. Nevertheless, this is an observational study that does not interfere with routine clinical practice; therefore, the study population was limited by the number of patients subjected to treatment with biological drugs. Furthermore, the variability inherent to ELISA techniques has resulted in the exclusion of outliers, leading to a reduction of the sample size.

In conclusion, as far as we know, this is the first study analysing histone modifications associated with drug response in psoriasis. Although significant differences in histone H3K27 methylation were found between responder and non-responder patients to biological drugs measured by PASI75, further studies performed in a larger subset are needed to confirm these results. However, these findings set the basis to develop our knowledge about pharmacoepigenetic biomarkers for the prediction of drug response in psoriasis.

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CONFLICT OF INTEREST

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 conflict of interests (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, Lilly and Celgene. M. Llamas Velasco has potential conflict of interests 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.

AUTHOR CONTRIBUTIONS

MCOB, AR, PSJ, MSR, EMA, MLV, SMV and TC performed research. MCOB and PSJ analysed data. MCOB wrote the paper. MR and DO contributed essential reagents/tools. ED and FAS designed the research study and critically reviewed the manuscript. All authors have read and approved the final manuscript.

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SUPPORTING INFORMATION

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

Figure S1 Boxplot representation of the global histone acetylation/methylation levels in PBMCs from psoriatic patients with or withou PsA and healthy controls. (A) Global histone H3 acetylation boxplot of psoriatic patients without PsA (n=28) and healthy controls (n=31). (B) Global histone H3 acetylation boxplot of psoriatic patients with PsA (n=11) and healthy controls (n=31). (C) Global histone H4 acetylation boxplot of psoriatic patients without PsA (n=26) and healthy controls (n=36). (D) Global histone H4 acetylation boxplot of psoriatic patients with PsA (n=11) and healthy controls (n=36). (E).

Histone H3K4 methylation boxplot of psoriatic patients without PsA (n=24) and healthy controls (n=42). (F) Histone H3K4 methylation boxplot of psoriatic patients with PsA (n=9) and healthy controls (n=42) (G). Histone H3K27 methylation boxplot of psoriatic patients without PsA (n=22) and healthy controls (n=40). (H) Histone H3K27 methylation boxplot of psoriatic patients with PsA (n=10) and healthy controls (n=40). The bottom of the box represents the 25th percentile while the top of the box represents the 75th percentile. Whiskers extend to more extreme values (1.5 times the interquartile range from the box). Circles represent outliers that do not fall in the whiskers while triangles represent extreme outliers (3 times the interquartile range from the box) (*, p<0.050). Abbreviation: PsA: psoriatic arthritis.

Figure S2 Boxplot representation of the global histone acetylation/methylation levels in PBMCs from psoriatic patients with and without PsA. (A) Global histone H3 acetylation boxplot of psoriatic patients without (n=28) and with PsA (n=11) (B). Global histone H4 acetylation boxplot of psoriatic patients without (n=26) and with PsA (n=11) (C). Histone H3K4 methylation boxplot of psoriatic patients without (n=24) and with PsA (n=9) (D). Histone H3K27 methylation boxplot of psoriatic patients without (n=22) and with PsA (n=10). The bottom of the box represents the 25th percentile while the top of the box represents the 75th percentile. Whiskers extend to more extreme values (1.5 times the interquartile range from the box). Circles represent outliers that do not fall in the whiskers while triangles represent extreme outliers (3 times the interquartile range from the box) (*, p<0.050). Abbreviation: PsA: psoriatic arthritis.

Figure S3 Boxplot representation of histone modifications before (baseline) and after treatment with biological drugs (final) in psoriatic patients without or with PsA. A) Global histone H3 acetylation boxplot in psoriatic patients without PsA (n=28). B) Global histone H3 acetylation boxplot in patients with PsA (n=11). C) Global histone H4 acetylation boxplot in patients without PsA (n=26). D) Global histone H4 acetylation boxplot in psoriatic patients without PsA (n=11). E) Histone H3K4 methylation boxplot (n=24) in psoriatic patients without PsA. F) Histone H3K4 methylation boxplot in psoriatic patients with PsA (n=9). G) Histone H3K27 methylation boxplot in psoriatic patients without PsA (n=22). H) Histone H3K27 methylation boxplot in psoriatic patients with PsA (n=10). The bottom of the box represents the 25th percentile while the top of the box represents the 75th percentile. Whiskers extend to more extreme values (1.5 times the interquartile range from the box). Circles represent outliers that do not fall in the whiskers while triangles represent extreme outliers (3 times the interquartile range from the box). Abbreviation: PsA: psoriatic arthritis.

Figure S4 Boxplot representation of the association of histone modifications with PASI75 at 3 months of treatment with biological drugs in patients with or without PsA. A) Comparison of the increments in global histone H3 acetylation after drugs treatment between patients without PsA who achieved PASI75 (n=23) or did not achieve PASI75 (n=5) at 3 months of treatment with biological drugs. B) Comparison of the increments in global histone H4 acetylation after drug treatment between patients without PsA who achieved PASI75 (n=22) or did not achieve PASI75 (n=4) at 3 months of treatment with biological drugs. C) Comparison of the increments in histone H3K4 methylation after drug treatment between patients without PsA who achieved PASI75 (n=20) or did not achieve PASI75 (n=4) at 3 months of treatment with biological drugs. D) Comparison of the increments in histone H3K27 methylation after drug treatment without PsA who achieved PASI75 (n=19) or did not achieve PASI75 (n=3) at 3 months of treatment with biological drugs. Data are shown as median followed by inter-quartile range (25th and 75th percentiles). The bottom of the box represents the 25th percentile while the top of the box represents the 75th percentile. Whiskers extend to more extreme values (1.5 times the interquartile range from the box). Circles represent outliers that do not fall in the whiskers while triangles represent extreme outliers (3 times the interquartile range from the box) (*, p<0.050). Abbreviations: Δ: Increment in histone modification between before and after treatment administration; PsA: Psoriatic arthritis, PASI: Psoriasis Area Severity Index; PASI75: a reduction of 75% from the baseline PASI.

Figure S5 Boxplot representation of the association of histone modifications with PASI75 at 6 months of treatment with biological drugs in patients with or without PsA. A) Comparison of the increments in global histone H3 acetylation after drugs treatment between patients without PsA who achieved PASI75 (n=23) or did not achieve PASI75 (n=5) at 6 months of treatment with biological drugs. B) Comparison of the increments in global histone H4 acetylation after drug treatment between patients without PsA who achieved PASI75 (n=21) or did not achieve PASI75 (n=5) at 6 months of treatment with biological drugs. C) Comparison of the increments in histone H3K4 methylation after drug treatment between patients without PsA who achieved PASI75 (n=19) or did not achieve PASI75 (n=5) at 6 months of treatment with biological drugs. D) Comparison of the increments in histone H3K27 methylation after drug treatment without PsA who achieved PASI75 (n=18) or did not achieve PASI75 (n=3) at 6 months of treatment with biological drugs. Data are shown as median followed by inter-quartile range (25th and 75th percentiles). The bottom of the box represents the 25th percentile while the top of the box represents the 75th percentile. Whiskers extend to more extreme values (1.5 times the interquartile range from the box). Circles represent outliers that do not fall in the whiskers while triangles represent extreme outliers (3 times the interquartile range from the box) (*, p<0.050). Abbreviations: Abbreviations: Δ: Increment in histone modification between before and after treatment administration; PsA: Psoriatic arthritis, PASI: Psoriasis Area Severity Index; PASI75: a reduction of 75% from the baseline PASI.

Figure S6 Boxplot representation of the association of histone modifications with PASI75 at 6 months of treatment with biological drugs. A) Comparison of the increments in global histone H3 acetylation after drugs treatment between patients who achieved PASI75

(n=33) or did not achieve PASI75 (n=6) at 6 months of treatment with biological drugs. B) Comparison of the increments in global histone H4 acetylation after drugs treatment between patients who achieved PASI75 (n=31) or did not achieve PASI75 (n=6) at 6 months of treatment with biological drugs. C) Comparison of the increments in histone H3K4 methylation after drugs treatment between patients who achieved PASI75 (n=27) or did not achieve PASI75 (n=6) at 6 months of treatment with biological drugs. D) Comparison of the increments in histone H3K27 methylation acetylation after drugs treatment between patients who achieved PASI75 (n=27) or did not achieve PASI75 (n=5) at 6 months of treatment with biological drugs. Data are shown as median followed by inter-quartile range (25th and 75th percentiles). The bottom of the box represents the 25th percentile while the top of the box represents the 75th percentile. Whiskers extend to more extreme values (1.5 times the interquartile range from the box). Circles represent outliers that do not fall in the whiskers while triangles represent extreme outliers (3 times the interquartile range from the box) (*, p<0.050). Abbreviations: Δ: Increment in histone modification between before and after treatment administration; PsA: Psoriatic arthritis, PASI: Psoriasis Area Severity Index; PASI75: a reduction of 75% from the baseline PASI.

Figure S7 Boxplot representation of the association of histone modifications with PASI90 at 3 months of treatment with biological drugs. A) Comparison of the increments in global histone H3 acetylation after drugs treatment between patients who achieved PASI90 (n=25) or did not achieve PASI90 (n=14) at 3 months of treatment with biological drugs. B) Comparison of the increments in global histone H4 acetylation after drugs treatment between patients who achieved PASI90 (n=25) or did not achieve PASI90 (n=12) at 3 months of treatment with biological drugs. C) Comparison of the increments in histone H3K4 methylation after drugs treatment between patients who achieved PASI90 (n=21) or did not achieve PASI90 (n=11) at 3 months of treatment with biological drugs. D) Comparison of the increments in histone H3K27 methylation acetylation after drugs treatment between patients who achieved PASI90 (n=21) or did not achieve PASI90 (n=12) at 3 months of treatment with biological drugs. Data are shown as median followed by inter-quartile range (25th and 75th percentiles). The bottom of the box represents the 25th percentile while the top of the box represents the 75th percentile. Whiskers extend to more extreme values (1.5 times the interquartile range from the box). Circles represent outliers that do not fall in the whiskers while triangles represent extreme outliers (3 times the interquartile range from the box) (*, p<0.050). Abbreviations: Δ: Increment in histone modification between before and after treatment administration; PsA: Psoriatic arthritis, PASI: Psoriasis Area Severity Index; PASI90: a reduction of 90% from the baseline PASI.

Figure S8 Boxplot representation of the association of histone modifications with PASI90 at 6 months of treatment with biological drugs. A) Comparison of the increments in global histone H3 acetylation after drugs treatment between patients who achieved PASI90 (n=28) or did not achieve PASI90 (n=9) at 6 months of treatment with biological drugs. B) Comparison of the increments in global histone H4 acetylation after drugs treatment between patients who achieved PASI90 (n=27) or did not achieve PASI90 (n=8) at 6 months of treatment with biological drugs. C) Comparison of the increments in histone H3K4 methylation after drugs treatment between patients who achieved PASI90 (n=24) or did not achieve PASI90 (n=8) at 6 months of treatment with biological drugs. D) Comparison of the increments in histone H3K27 methylation acetylation after drugs treatment between patients who achieved PASI90 (n=23) or did not achieve PASI90 (n=7) at 6 months of treatment with biological drugs. Data are shown as median followed by inter-quartile range (25th and 75th percentiles). The bottom of the box represents the 25th percentile while the top of the box represents the 75th percentile. Whiskers extend to more extreme values (1.5 times the interquartile range from the box). Circles represent outliers that do not fall in the whiskers while triangles represent extreme outliers (3 times the interquartile range from the box) (*, p<0.050) Abbreviations: Δ: Increment in histone modification between before and after treatment administration; PsA: Psoriatic arthritis, PASI: Psoriasis Area Severity Index; PASI90: a reduction of 90% from the baseline PASI.

Figure S9 Boxplot representation of the association of histone modifications with PASI90 at 3 months of treatment with biological drugs in patients without PsA. A) Comparison of the increments in global histone H3 acetylation after drugs treatment between patients without PsA who achieved PASI90 (n=19) or did not achieve PASI90 (n=9) at 3 months of treatment with biological drugs. B) Comparison of the increments in global histone H4 acetylation after drug treatment between patients without PsA who achieved PASI90 (n=19) or did not achieve PASI90 (n=7) at 3 months of treatment with biological drugs. C) Comparison of the increments in histone H3K4 methylation after drug treatment between patients without PsA who achieved PASI90 (n=17) or did not achieve PASI90 (n=7) at 3 months of treatment with biological drugs. D) Comparison of the increments in histone H3K27 methylation after drug treatment without PsA who achieved PASI90 (n=16) or did not achieve PASI90 (n=6) at 3 months of treatment with biological drugs. Data are shown as median followed by inter-quartile range (25th and 75th percentiles). The bottom of the box represents the 25th percentile while the top of the box represents the 75th percentile. Whiskers extend to more extreme values (1.5 times the interquartile range from the box). Circles represent outliers that do not fall in the whiskers while triangles represent extreme outliers (3 times the interquartile range from the box) (*, p<0.050). Abbreviations: Δ: Increment in histone modification between before and after treatment administration; PsA: Psoriatic arthritis, PASI: Psoriasis Area Severity Index; PASI90: a reduction of 90% from the baseline PASI.

Figure S10 Boxplot representation of the association of histone modifications with PASI90 at 6 months of treatment with biological drugs in patients without PsA. A) Comparison of the increments in global histone H3 acetylation after drugs treatment between

patients without PsA who achieved PASI90 (n=19) or did not achieve PASI90 (n=8) at 6 months of treatment with biological drugs. B) Comparison of the increments in global histone H4 acetylation after drug treatment between patients without PsA who achieved PASI90 (n=18) or did not achieve PASI90 (n=7) at 6 months of treatment with biological drugs. C) Comparison of the increments in histone H3K4 methylation after drug treatment between patients without PsA who achieved PASI90 (n=16) or did not achieve PASI90 (n=7) at 6 months of treatment with biological drugs. D) Comparison of the increments in histone H3K27 methylation after drug treatment without PsA who achieved PASI90 (n=15) or did not achieve PASI90 (n=5) at 6 months of treatment with biological drugs. Data are shown as median followed by inter-quartile range (25th and 75th percentiles). The bottom of the box represents the 25th percentile while the top of the box represents the 75th percentile. Whiskers extend to more extreme values (1.5 times the interquartile range from the box). Circles represent outliers that do not fall in the whiskers while triangles represent extreme outliers (3 times the interquartile range from the box) (*, $p<0.050$). Abbreviations: Δ: Increment in histone modification between before and after treatment administration. Psoriatic arthritis (PsA), PASI (Psoriasis Area Severity Index); PASI90 (a reduction of 90% from the baseline PASI).

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