Journal of Autoimmunity 102 (2019) 126–132

Contents lists available at ScienceDirect

Journal of Autoimmunity

journal homepage: www.elsevier.com/locate/jautimm

Genome-wide DNA methylation analysis in ankylosing spondylitis identifies *HLA-B*27* dependent and independent DNA methylation changes in whole blood

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ARTICLE INFO

Keywords:
Ankylosing spondylitis
Epigenetics
Methylation
HLA-B*27

ABSTRACT

Background and objective: Ankylosing spondylitis is a chronic inflammatory disease characterized by inflammation of the sacroiliac joints and the spine that can lead to significant pain, immobility, and disability. The etiology and pathogenesis of ankylosing spondylitis are incompletely understood, though most patients carry the *HLA-B*27* allele. The objective of this study was to evaluate DNA methylation changes in ankylosing spondylitis with the goal of revealing novel mechanistic insights into this disease.

Methods: Genome-wide DNA methylation analysis was performed in whole blood DNA samples using the Infinium MethylationEPIC array in patients with ankylosing spondylitis compared to age, sex, and race matched patients with osteoarthritis as a non-inflammatory disease control. We studied 24 patients with ankylosing spondylitis, including 12 patients who carry *HLA-B*27* and 12 patients who are *HLA-B*27* negative. DNA methylation analysis was performed with adjustment for blood cell composition in each sample.

Results: We identified a total of 67 differentially methylated sites between ankylosing spondylitis patients and osteoarthritis controls. Hypermethylated genes found included GTPase-related genes, while hypomethylated genes included *HCP5*, which encodes a lncRNA within the MHC region, previously associated with genetic risk for psoriasis and toxic epidermal necrolysis. Carrying *HLA-B*27* was associated with robust hypomethylation of *HCP5*, tubulin folding cofactor A (*TBCA*) and phospholipase D Family Member 6 (*PLD6*) in ankylosing spondylitis patients. Hypomethylation within *HCP5* involves a CpG site that contains a single nucleotide polymorphism in linkage disequilibrium with *HLA-B*27* and that controls DNA methylation at this locus in an allele-specific manner.

Conclusions: A genome-wide DNA methylation analysis in ankylosing spondylitis identified DNA methylation patterns that could provide potential novel insights into this disease. Our findings suggest that *HLA-B*27* might play a role in ankylosing spondylitis in part through inducing epigenetic dysregulation.

1. Introduction

Ankylosing spondylitis (AS) is a spondyloarthritis of unknown etiology characterized by inflammation of the axial joints, including the sacroiliac joints and the spine, that can lead to significant pain, immobility, and disability [1]. The major histocompatibility complex I allele *HLA-B*27* is the strongest genetic risk factor for AS [2]. Though the genetic association of *HLA-B*27* and AS is robust, it is not the sole

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https://doi.org/10.1016/j.jaut.2019.04.022
Received 4 March 2019; Received in revised form 28 April 2019; Accepted 29 April 2019
Available online 23 May 2019
0896-8411/

factor in disease pathogenesis as only about 5% of *HLA-B*27* carriers will develop spondyloarthritis [3]. Genome-wide association studies have identified additional risk loci including in genes associated with the IL-23-IL-17 pathway [1]. Environmental factors that may contribute to the development of AS include the interaction of host genetics and their microbiome, and chemical exposures including smoking [3–6]. These interactions might result in changes in epigenetic regulation including DNA methylation, microRNAs, and histone modifications. Prior work has identified DNA methylation changes at both the gene-specific and genome-wide levels in AS patients [7–9]. To date, there have been no studies examining the effect of *HLA-B*27* status on DNA methylation within the genome of AS patients.

In this study, we aimed to identify DNA methylation change in AS patients compared to controls, and examine the effect of *HLA-B*27* on DNA methylation patterns in AS patients.

2. Materials & methods

2.1. Study participants and demographics

Twenty four AS patients (12 *HLA-B*27* positive and 12 *HLA-B*27* negative) and 12 osteoarthritis (OA) disease controls were enrolled in this study. All patients in this study were European-American males, and they were selected to be matched for age as much as possible within the 3 groups. Patients were recruited and enrolled at routine clinic visits and assessed by their treating rheumatologist for modified New York Classification Criteria (1984) and Assessment of Spondyloarthritis International Society Classification Criteria (2009) [10,11]. Patients were recruited from the Albany Stratton VAMC (Albany, NY), Rocky Mountain Regional VAMC (Aurora, CO), George E. Wahlen VAMC (Salt Lake, UT) and G.V. (Sonny) Montgomery VAMC (Jackson, MS) as part of the Program to Understand the Longterm Outcomes in Spondyloarthritis (PULSAR). The rationale for including OA patients as control in this study is to increase the specificity of methylation changes we identify in AS, and reduce the likelihood of detecting methylation changes secondary to non-specific joint damage. The institutional review boards of each institution and the PULSAR Scientific and Ethics Advisory Committee approved this study and all patients signed informed consent prior to study enrollment.

2.2. Whole blood collection, DNA isolation and HLA-B*27 genotyping

Whole blood was collected in EDTA-containing tubes from each patient during PULSAR enrollment visits. DNA was extracted using the Qiagen DNeasy Blood and Tissue Kit (Germantown, MD, USA) and stored at −20 °C until the time of DNA methylation analysis. *HLA-B*27* genotyping was performed in the clinical laboratory at each PULSAR site using polymerase chain reaction (PCR)/sequence-specific oligonucleotide probes (LabCorp, Burlington, NC, USA) or antigen-antibody binding and flow cytometry (Quest Diagnostics, Secaucus, NY, USA). OA subjects were not tested for *HLA-B*27*.

2.3. DNA methylation studies

Genomic DNA from each patient was bisulfite converted using the EZ DNA Methylation Kit (Zymo Research, Irvine, CA, USA) according to manufacturer's directions for use with the Illumina DNA methylation arrays. Bisulfite-converted DNA was hybridized to Infinium MethylationEPIC arrays (Illumina, San Diego, CA, USA) to measure site-specific DNA methylation in over 850,000 methylation sites across the genome. All DNA methylation array processing was performed at the University of Michigan DNA Sequencing Core.

2.4. Data analysis

IDAT files containing probe-wise methylated and unmethylated channel values for each sample were loaded into *minfi* (v1.24) in the R statistical environment (v3.4.2) for data cleaning, normalization, and calculating methylation values. All samples had > 99% of probes with a detection *P*-value < 0.01 and a median log methylated and unmethylated value > 10.5. Methylation values were normalized using stratified quantile normalization [12]. This procedure stratifies probes into groups based on their genomic region and quantile normalizes type II probes first then uses their distribution as a reference for normalizing type I probes. Methylated and unmethylated values for each probe were used to calculate an average methylation value (β) for each sample.

Prior to statistical testing, we filtered probes that were likely technical artifacts. Probes that met any of these three criteria were removed: a detection *P*-value > 0.01, the presence of a SNP within 10bp of the 3′ end of the probe with a minor allele frequency ≥5%, or previously identified as a cross-reactive probes (Supplemental Table 1 of Pidsley et al. 2016) [13]. We used the 'estimateCellCounts' command in *minfi* to calculate an estimated cell type composition of our whole blood samples *in silico* [14,15]. Prior to regression analysis, we squeezed the data using the formula (β-0.5)*0.99 + 0.5 which is recommended for beta regression [16]. Beta regression was used to model the correlation between CpG methylation and either disease or *HLA-B*27* status. Illumina array ID and percentage of CD8 T cells, CD4 T cells, NK cells, B cells, monocytes, and granulocytes were included as covariates for adjustment. Multiple-testing correction was done by calculating the Benjamini-Hochberg FDR-adjusted *P*-values for each probe with a cutoff of < 0.05. Regression analysis was conducted using the *betareg* (v3.1) package in R [17,18].

Statistical testing for age and smoking status were conducted using GraphPad Prism 8 (San Diego, CA, USA). *P*-values < 0.05 were considered significant.

2.5. Gene ontology term analysis

Gene ontology (GO) term analysis was performed using *Enrichr* [19,20]. Gene set enrichment was calculated using a Fisher's exact test that compares proportions assuming a binomial distribution and an independent probability of any gene belonging to any gene set. *P*-values were adjusted for multiple testing using the Benjamini-Hochberg method. Z-scores were calculated by first performing Fisher's exact test of many random input gene lists to compute a mean rank and standard deviation expected for each term. The expected rank for each term is compared to actual rank and the deviation in the z-score. We selected 'Molecular Function' and 'Biological Process' gene ontology families to contextualize our results. Results were considered significant if their FDR-adjusted *P*-value was < 0.05.

2.6. Sanger sequencing of CpG-SNPs

The SNP rs114212906 (cg17616250; Forward: 5′-TTGCCTCATGCC AAGAAAAT-3'; Reverse: 5′-CAGACACCTCTTTCAGCCTGT-3′) in the *HCP5* locus was amplified using ZymoTaq DNA Polymerase (Zymo Research, Irvine, CA, USA). Sanger sequencing was used to determine the genotype and confirm rs114212906 as CpG-SNP in 11 *HLA-B*27* positive and 12 *HLA-B*27* negative AS patients.

3. Results

3.1. Patient population

AS patients and OA controls were matched for age, sex, and race. There was no significant difference in the mean age of patients between the groups (mean age ± SD) (*HLA-B*27* positive: 58.1 ± 15.0; *HLA-B*27* negative: 55.5 ± 14.8; OA: 57.0 ± 18.0) (One-way ANOVA ($F_{(2,33)} = 0.08$); $P = 0.92$). Smoking status (current or former/non-smoking) was independent of patient group (X^2 (1; N = 35) = 0.26; P = 0.61). Patients demographics, smoking status, and medications used at the time of enrollment in our study are shown in Supplementary Table 1.

Table 1
Top 10 hypomethylated and top 10 hypermethylated sites in AS vs OA and (left), and *HLA-B*27* positive vs *HLA-B*27* negative AS patients (right). These sites met a threshold of Δβ > 10% or < −10% and FDR-adjusted *P*-value < 0.05. AS: ankylosing spondylitis; OA: osteoarthritis.

CG ID	Location (hg19)	Gene Symbol (GENCODE V12)	Methylation Fraction (β)			Fold Change (AS/OA)	FDR-adjusted P-value	CG ID	Location (hg19)	Gene Symbol (GENCODE V12)	Methylation Fraction (β)			Fold Change (+/-)	FDR-adjusted P-value
			AS	OA	Δβ						*HLA-B*27 +*	*HLA-B*27 -*	Δβ		
cg17616250	6:31391389	*HCP5*	0.50	0.76	-0.26	0.66	1.51E-03	cg17616250	6:31391389	*HCP5*	0.41	0.60	-0.19	0.68	2.38E-04
cg12313868	11:30338751	-	0.18	0.38	-0.20	0.47	3.66E-05	cg11012412	5:77147141	*TBCA*	0.33	0.52	-0.19	0.64	5.15E-08
cg11102724	5:150619039	-	0.13	0.32	-0.19	0.40	1.78E-02	cg14167415	5:77146999	*TBCA*	0.47	0.66	-0.18	0.72	5.11E-06
cg00299286	5:180478185	-	0.24	0.43	-0.19	0.56	1.56E-05	cg26539818	17:17109691	*PLD6*	0.13	0.31	-0.18	0.43	1.81E-07
cg03192273	5:150618948	-	0.10	0.27	-0.17	0.36	1.06E-02	cg01412970	17:17109239	*PLD6*	0.33	0.50	-0.17	0.66	4.26E-08
cg24875593	21:45153009	*PDXK*	0.69	0.86	-0.17	0.81	2.29E-03	cg03726259	16:2358313	*ABCA3*	0.64	0.80	-0.16	0.80	3.71E-11
cg11671940	10:28781637	*RP11-351M16.3*	0.31	0.46	-0.14	0.68	2.39E-02	cg25438517	5:77146796	*TBCA*	0.57	0.73	-0.16	0.78	9.80E-06
cg20787649	1:17636898	-	0.47	0.62	-0.14	0.77	1.55E-03	cg08779649	13:50194554	-	0.39	0.55	-0.15	0.72	5.99E-05
cg21055985	3:78921616	-	0.36	0.51	-0.14	0.72	3.20E-02	cg10064060	16:15603154	*RP11-1021N1.1;C16orf45*	0.39	0.55	-0.15	0.72	1.44E-05
cg02095219	17:57976723	RPS6KB1	0.53	0.67	-0.14	0.79	2.37E-02	cg02827245	2:118730140	CCDC93	0.60	0.75	-0.15	0.80	8.51E-12
cg27237671	2:676223	*TMEM18;AC092159.2*	0.53	0.39	0.14	1.35	3.77E-02	cg22401033	19:3041700	-	0.79	0.63	0.17	1.27	2.78E-02
cg24141001	17:34774445	*U6;TBC1D3H*	0.78	0.65	0.14	1.21	4.15E-02	cg05276469	17:16570473	-	0.33	0.16	0.17	2.04	2.79E-02
cg16301894	4:129389744	*RP11-420A23.1*	0.48	0.34	0.14	1.41	6.32E-03	cg04578890	20:1349052	*FKBP1A-SDCBP2*	0.79	0.63	0.17	1.27	1.78E-03
cg26292116	2:241618820	*AQP12B*	0.48	0.34	0.14	1.41	2.61E-02	cg23053444	14:78471899	-	0.48	0.31	0.17	1.56	7.55E-03
cg21028319	8:38847958	*TM2D2*	0.58	0.44	0.15	1.34	1.54E-02	cg26587553	12:93277453	*EEA1*	0.65	0.47	0.17	1.37	8.11E-03
cg24354335	1:23291783	*LACTBL1*	0.56	0.41	0.15	1.37	2.39E-05	cg17939448	4:77200832	*FAM47E;STBD1*	0.52	0.34	0.18	1.52	5.92E-06
cg02872767	19:1525453	-	0.76	0.61	0.15	1.25	4.61E-04	cg15991630	18:77292555	-	0.62	0.43	0.18	1.43	1.40E-02
cg11789449	1:160033113	*KCNJ10;RP11-536C5.2*	0.66	0.47	0.19	1.41	7.83E-03	cg01871025	X:76648652	-	0.77	0.57	0.20	1.36	3.17E-04
cg07355270	X:11211491	*ARHGAP6*	0.69	0.46	0.23	1.49	2.85E-02	cg23075597	12:133322256	*ANKLE2*	0.82	0.60	0.22	1.37	1.30E-07
cg08477332	1:153590243	*S100A14*	0.52	0.25	0.27	2.05	3.43E-02	cg02006792	X:24295207	-	0.52	0.26	0.26	2.00	1.57E-02

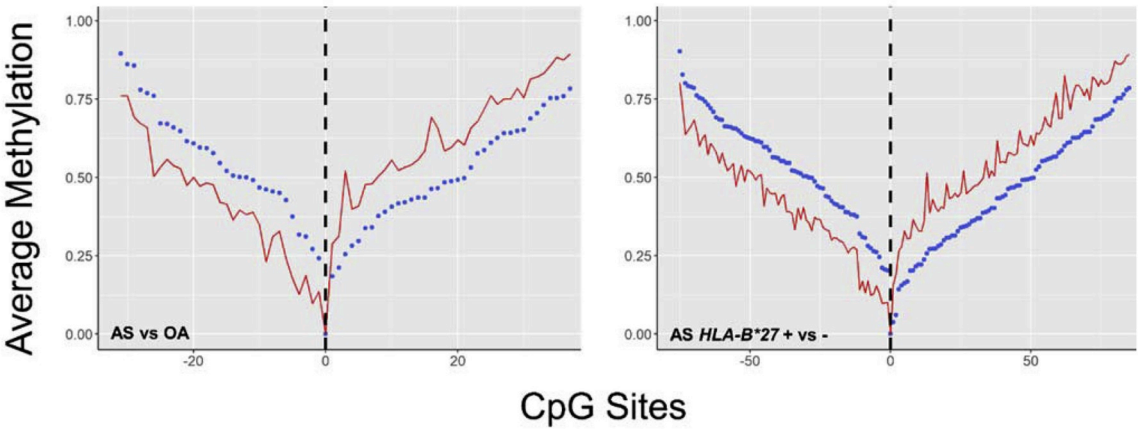

Fig. 1. Hypomethylated (left of dotted vertical line) and hypermethylated (right of dotted vertical line) sites in AS vs OA and *HLA-B*27* positive vs *HLA-B*27* negative AS patients. Red lines represent the average methylation of all AS or *HLA-B*27* positive AS patients, respectively. Blue dots represent the average methylation of all OA or *HLA-B*27* negative AS patients, respectively. These methylation sites met a threshold of $\Delta\beta > 10\%$ or $< -10\%$ and FDR-adjusted *P*-value < 0.05. AS: ankylosing spondylitis; OA: osteoarthritis.

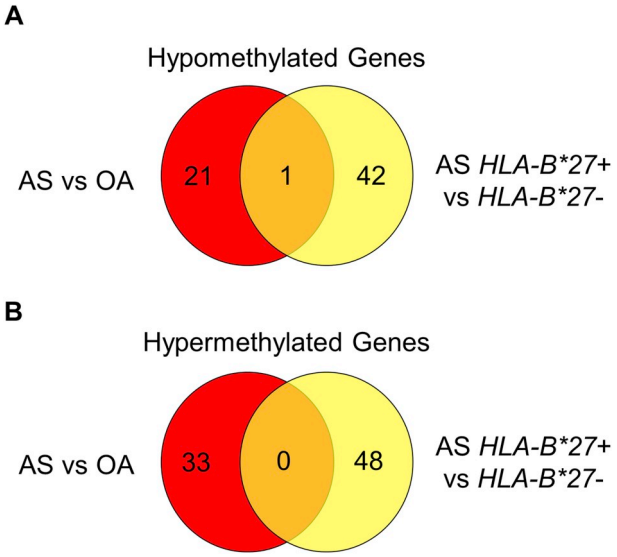

Fig. 2. Venn diagrams revealing the number of unique and shared differentially methylated genes between AS vs OA (left circles) and *HLA-B*27* positive vs *HLA-B*27* negative AS patients (right circles). Hypomethylated and hypermethylated genes are depicted in panels A and B, respectively. AS: ankylosing spondylitis; OA: osteoarthritis.

3.2. Global DNA methylation changes specific to ankylosing spondylitis and HLA-B*27 status

Our goal was to identify differentially methylated CpG sites in AS that are associated with *HLA-B*27* status. We first compared AS patients and OA disease controls. We found 30 hypomethylated and 37 hypermethylated CpG sites in AS patients compared to controls with a methylation difference of at least 10% and an FDR-adjusted *P*-value < 0.05. Hypomethylated and hypermethylated CpG sites were annotated to 22 and 33 genes, respectively (GENCODE Complete V12) (Table 1 and Supplementary Table 2). We then stratified AS patients based on *HLA-B*27* status and identified 74 hypomethylated and 85 hypermethylated CpG sites in *HLA-B*27* positive compared to *HLA-B*27* negative patients with a methylation difference of at least 10% and an FDR-adjusted *P*-value < 0.05. Hypomethylated and hypermethylated CpG sites were annotated to 43 and 48 genes, respectively (GENCODE Complete V12) (Fig. 1, Table 1, and Supplementary Table 3).

Genes hypermethylated in AS patients were significantly enriched for molecular function GO terms that included GTPase activator activity (GO:0005096), GTPase regulator activity (GO:0030695), and Rab/Ras GTPase binding (GO:0017137 & GO:0017016) (Supplementary Table 4). Genes hypermethylated in *HLA-B*27* positive compared to *HLA-B*27* negative AS patients were enriched for potassium ion binding and alkali metal ion binding molecular function GO terms (GO:0030955 & GO:0031420) (Supplementary Table 5). Hypomethylated genes in both analyses showed no significant gene set enrichments after adjusting for multiple testing.

The methylation site cg17616250, which was annotated to the HLA Complex P5 RNA gene *HCP5* was the only gene-annotated CpG site differentially methylated in both analyses comparing AS patients to OA controls and comparing *HLA-B*27* positive to *HLA-B*27* negative AS patients (Fig. 2). It was also the most hypomethylated site in both analyses. The average methylation of cg17616250 in AS patients (50%) was lower compared to OA patients (76%) (*P-value* = 1.51E-03) (Fig. 3A). The average methylation in *HLA-B*27* positive AS patients (41%) was significantly lower compared to *HLA-B*27* negative patients (60%) (*P*-value = 2.38E-04) (Fig. 3B).

3.3. DNA methylation of cg17616250 is directly linked to HLA-B*27 status by a CpG-SNP

A single nucleotide polymorphism rs114212906(C/T) was shown to exist in the CpG site (cg17616250, chr6:31391389; hg19) [21]. To confirm this, we used Sanger sequencing to determine the genotype of eleven *HLA-B*27* positive and twelve *HLA-B*27* negative AS patients. Ten *HLA-B*27* positive and seven *HLA-B*27* negative AS patients were heterozygous for the minor "T" allele. TpG sites do not get methylated so a heterozygous carrier could only have 50% of the methylation of a homozygous "C" carrier. One *HLA-B*27* positive and none of the *HLA-B*27* negative AS patients were homozygous for the "T" allele. None of the *HLA-B*27* positive and five *HLA-B*27* negative AS patients were homozygous for the "C" allele (Fig. 3C). These data indicate that the methylation status of cg17616250 in *HCP5* is determined by the genotype in rs114212906 (ANOVA F (2, 20) = 791.1; P < 0.0001). This SNP is in high linkage disequilibrium with rs4349859 ($R^2 = 0.91$; HaploReg v4.1), which has been previously reported to tag *HLA-B*27* in AS patients [22,23]. This confirms that the genetic variant present in the CpG methylation site in *HCP5*, and that determined its methylation status in our study, is linked to *HLA-B*27* status in AS patients.

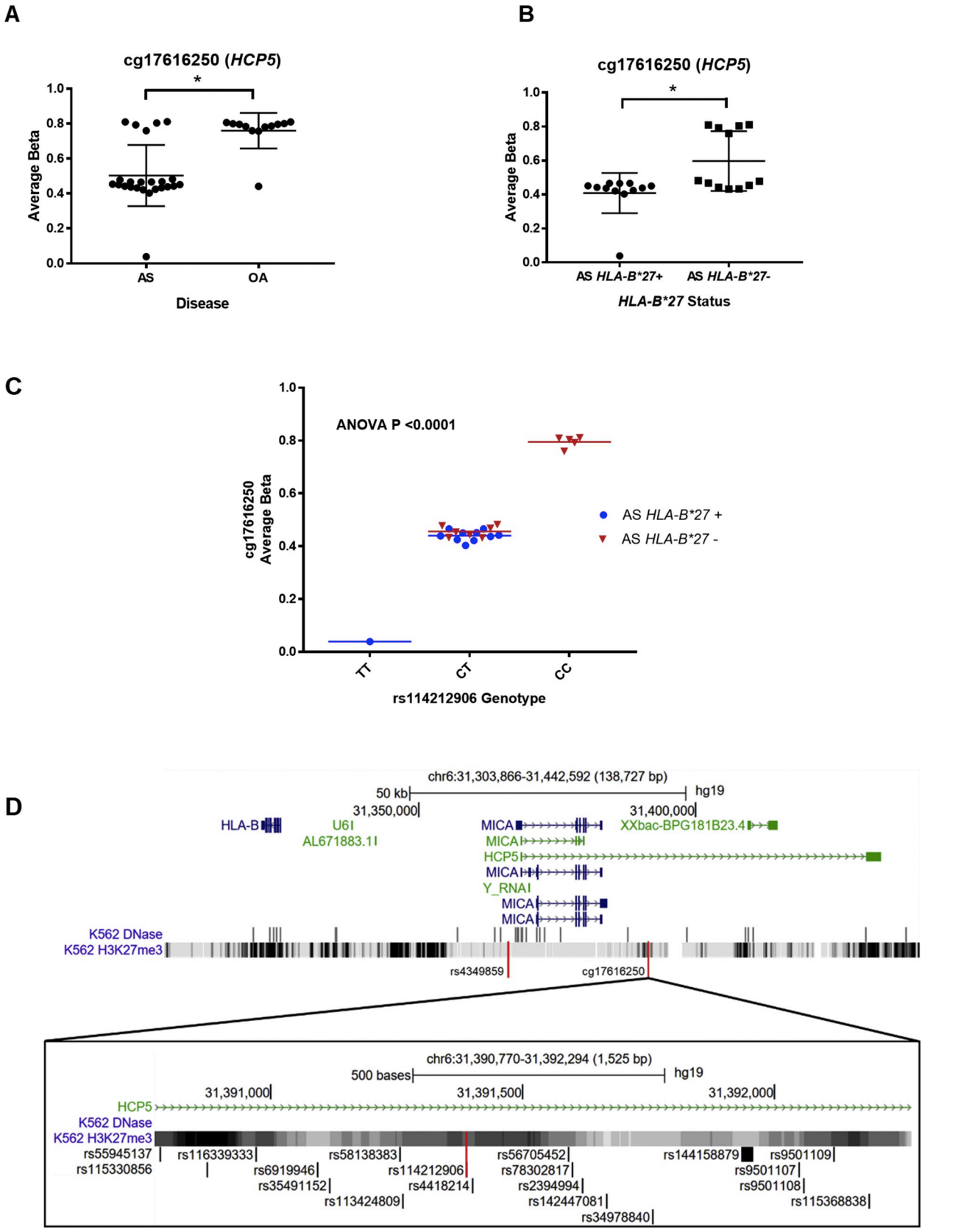

Fig. 3. Differential methylation of cg17616250 (*HCP5*) categorized by (A) disease and (B) *HLA-B*27* status. (C) Average methylation of *HLA-B*27* positive and *HLA-B*27* negative AS patients showed correlation between rs114212906 genotype and *HLA-B*27* status (negative = red triangles, positive = blue circles). (D) The genomic region surrounding the CpG-SNP rs114212906. Rs114212906 is in high linkage disequilibrium with rs4349859 that tags *HLA-B*27* in ankylosing spondylitis patients. Rs114212906 overlaps a region of H3K27me3 in K562 leukemic cell line and lacks DNase hypersensitivity indicating a region of heterochromatin. AS: ankylosing spondylitis; OA: osteoarthritis.

4. Discussion

Ankylosing spondylitis is robustly associated with *HLA-B*27*, however, the mechanisms through which this allele increases the risk of ankylosing spondylitis remain poorly understood. Proposed mechanisms include altered processing and presentation of antigen or surface expression of HLA-B*27 dimers that lead to a skewed T cell response and ultimately disease [2]. Our results suggest that *HLA-B*27* could potentially have a pathophysiologic effect by influencing the DNA methylation of CpG sites surrounding the *HLA-B* locus in an allele-specific manner.

Previous studies suggested that PBMCs isolated from AS patients have increased promoter methylation and decreased expression of the DNA methyltransferase 1 gene, *DNMT1*, which maintains CpG methylation [7]. This could result in genome-wide DNA methylation changes. The B-cell chronic lymphocytic leukemia/lymphoma 11B gene *BCL11B* also showed promoter hypermethylation in PBMCs and reduced gene expression in AS patients [9]. In whole blood, we did not detect DNA methylation differences in *BCL11B*, but we did detect hypermethylation of a single CpG site in the related gene *BCL11A* (cg10610477; $\Delta\beta = 0.14$; FDR-adjusted *P*-value = 5.07E-03) in *HLA-B*27* positive compared to *HLA-B*27* negative AS patients.

Genome-wide DNA methylation changes associated with AS have been previously identified in a study of Chinese patients, which found significant hypermethylation of a CpG site in *HLA-DQB1* in AS patients and decreased *HLA-DQB1* mRNA expression in PBMCs [8]. The same study also revealed differential methylation of genes involved in several inflammatory and autoimmune pathways and MHC class II antigen presentation. Our study did not replicate the finding of hypermethylation of *HLA-DQB1* in AS patients or detect the same gene pathways. This might be due to differences in the genetic background between the patients enrolled in the two studies. Although our study was performed in whole blood, we did adjust for estimated cell type composition.

We noted the consistent and extensive hypomethylation of tubulin folding cofactor A gene *TBCA* and phospholipase D family member 6 gene *PLD6* at several CpG sites in *HLA-B*27* positive compared to *HLA-B*27* negative AS patients. In *TBCA*, differentially methylated CpG sites extended across the north and south shores, south shelf, and body of a CpG island, all within the 5' untranslated region. In *PLD6*, CpG sites extended across a CpG island that included the region 200bp upstream of the transcription start site and first exon of the gene. CpG islands are regions of the genome with a greater density of CpG sites than expected, and are predominantly unmethylated [24]. Almost 50% of CpG islands are located near the transcription start site of genes and have been associated with regulating gene transcription through histone modifications, DNA methylation, and altering chromatin accessibility [25]. TBCA is a β-tubulin chaperone protein that is involved in microtubule formation in cells [26]. TBCA expression is required for cell viability, cytoskeletal regulation, and differentiation [26]. PLD6 (MitoPLD) is a member of the phospholipase D family of enzymes that produces phosphatidic acid that is uniquely expressed on the surface of mitochondria and is involved in the process of mitochondrial fusion [27]. Neither gene has previously been identified as being differentially methylated in ankylosing spondylitis patients. Our data suggest an interaction between *HLA-B*27* and hypomethylation in *TBCA* and *PLD6*, however, the mechanism and possible consequences of this interaction remain to be examined.

Currently it is unknown what, if any, effect rs114212906 has on regulating nearby genes through limiting DNA methylation of cg17616250. *HCP5* is an RNA gene that shares sequence homology with endogenous human retroviruses and is highly expressed in lymphocytes [28,29]. Cg17616250 is not located in or near a CpG island, but is located in an H3K27me3 region identified in the K562 leukemic cell line from ENCODE (Fig. 3D) [30]. H3K27me3 is a histone modification that indicates heterochromatin and transcriptional repression [31]. CpG-SNPs represent genetic variants that alter CpG sites and therefore methylation levels. They represent the majority of local (< 1 kb in distance) methylation quantitative trait loci (meQTL) and are enriched in regions of inactive chromatin in blood cells [32]. Genetic variants in CpG-SNPs can be in high linkage disequilibrium (LD) with other genetic variants within haplotype blocks, making the methylation change a tag for other genetic polymorphisms. In this study we identified a differentially methylated CpG-SNP in LD with *HLA-B*27*, making this methylation change potentially relevant to the pathogenesis of AS and the disease risk conferred by *HLA-B*27*. Allele-specific regulatory changes can alter the recruitment of methyl-binding domain proteins that help regulate chromatin architecture or modify the binding site of transcription factors, resulting in downstream gene expression differences [33].

A recent study used publicly available histone modification data and gene and protein expression from various tissues to identify the cell types most impacted by risk variants in AS patients [34]. The study concluded that immune cell types including $CD4^+$ and $CD8^+$ T cells, monocytes, NK cells, and gut tissue were likely to be relevant to AS pathogenesis. Similar tissue-specific studies of DNA methylation will give a clearer picture of how genetic variants associated with AS impact DNA methylation and gene expression patterns in specific disease-relevant cell types.

Strengths of our study include that both *HLA-B*27* positive and negative AS patients matched for age, sex, and race were included. Our data, however, may not be generalizable to women or AS patients of non-European ancestry. An additional limitation is that OA patients were not tested for *HLA-B*27*, although based on the frequency of *HLA-B*27* in European populations, we expect that the vast majority were *HLA-B*27* negative. Moreover, which specific cell subsets or tissues are influenced at the transcriptional level by the *HLA-B*27*-associated methylation changes we identified, and how they might affect the immune response or induce possible pathogenic consequences relevant to ankylosing spondylitis remains to be studied.

5. Conclusions

In this study, we describe for the first time a direct association between *HLA-B*27* and changes in DNA methylation in AS patients. This includes the presence of a CpG-SNP that provides a direct mechanistic link between the genetic background and DNA methylation in this disease. Understanding the extent of allele-specific DNA methylation changes and their impact will require tissue-specific methylation and expression sequencing of genes in the MHC-I region in future studies. Our novel findings suggest that the contribution of *HLA-B*27* to disease pathogenesis in ankylosing spondylitis could include direct changes to the DNA methylation status of the surrounding region (such as in *HCP5*), or in other potentially disease-relevant genetic loci such as in *TBCA* and *PLD6*.

Conflicts of interest

None of the authors has any financial conflict of interest with the work presented.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jaut.2019.04.022.

References

[1] J.D. Taurog, A. Chhabra, R.A. Colbert, Ankylosing Spondylitis, Axial Spondyloarthritis, N. Engl. J. Med. 374 (2016) 2563–2574.
[2] B. Chen, J. Li, C. He, D. Li, W. Tong, Y. Zou, et al., Role of HLA-B27 in the pathogenesis of ankylosing spondylitis (Review), Mol. Med. Rep. 15 (2017) 1943–1951.
[3] P. Kaushik, Epigenetics and axial spondyloarthritis - DNA is not destiny, J. Clin.

Epigenet. 03 (2017).
[4] I. Shiue, Relationship of environmental exposures and ankylosing spondylitis and spinal mobility: US NHAENS, 2009-2010, Int. J. Environ. Health Res. 25 (2015) 322–329.
[5] M. Asquith, J.T. Rosenbaum, The interaction between host genetics and the microbiome in the pathogenesis of spondyloarthropathies, Curr. Opin. Rheumatol. 28 (2016) 405–412.
[6] V. Videm, A. Cortes, R. Thomas, M.A. Brown, Current smoking is associated with incident ankylosing spondylitis – the HUNT population-based Norwegian health study, J. Rheumatol. 41 (2014) 2041–2048.
[7] S. Aslani, M. Mahmoudi, M. Garshasbi, A.R. Jamshidi, J. Karami, M.H. Nicknam, Evaluation of DNMT1 gene expression profile and methylation of its promoter region in patients with ankylosing spondylitis, Clin. Rheumatol. 35 (2016) 2723–2731.
[8] J. Hao, Y. Liu, J. Xu, W. Wang, Y. Wen, A. He, et al., Genome-wide DNA methylation profile analysis identifies differentially methylated loci associated with ankylosis spondylitis, Arthritis Res. Ther. 19 (2017) 177.
[9] J. Karami, M. Mahmoudi, A. Amirzargar, M. Gharshasbi, A. Jamshidi, S. Aslani, et al., Promoter hypermethylation of BCL11B gene correlates with downregulation of gene transcription in ankylosing spondylitis patients, Genes Immun. 18 (2017) 170–175.
[10] S. van der Linden, H.A. Valkenburg, A. Cats, Evaluation of diagnostic criteria for ankylosing spondylitis. A proposal for modification of the New York criteria, Arthritis Rheum. 27 (1984) 361–368.
[11] M. Rudwaleit, R. Landewe, D. van der Heijde, J. Listing, J. Brandt, J. Braun, et al., The development of Assessment of SpondyloArthritis international Society classification criteria for axial spondyloarthritis (part I): classification of paper patients by expert opinion including uncertainty appraisal, Ann. Rheum. Dis. 68 (2009) 770–776.
[12] N. Touleimat, J. Tost, Complete pipeline for Infinium((R)) Human Methylation 450K BeadChip data processing using subset quantile normalization for accurate DNA methylation estimation, Epigenomics 4 (2012) 325–341.
[13] R. Pidsley, E. Zotenko, T.J. Peters, M.G. Lawrence, G.P. Risbridger, P. Molloy, et al., Critical evaluation of the Illumina MethylationEPIC BeadChip microarray for whole-genome DNA methylation profiling, Genome Biol. 17 (2016) 208.
[14] E.A. Houseman, W.P. Accomando, D.C. Koestler, B.C. Christensen, C.J. Marsit, H.H. Nelson, et al., DNA methylation arrays as surrogate measures of cell mixture distribution, BMC Bioinf. 13 (2012) 86.
[15] A.E. Jaffe, R.A. Irizarry, Accounting for cellular heterogeneity is critical in epigenome-wide association studies, Genome Biol. 15 (2014) R31.
[16] M. Smithson, J. Verkuilen, A better lemon squeezer? Maximum-likelihood regression with beta-distributed dependent variables, Psychol. Methods 11 (2006) 54–71.
[17] F. Cribari-Neto, A. Zeileis, Beta regression inR, J. Stat. Softw. 34 (2010).
[18] B. Grün, I. Kosmidis, A. Zeileis, Extended beta regression inR: shaken, stirred, mixed, and partitioned, J. Stat. Softw. 48 (2012).
[19] E.Y. Chen, C.M. Tan, Y. Kou, Q. Duan, Z. Wang, G.V. Meirelles, et al., Enrichr: interactive and collaborative HTML5 gene list enrichment analysis tool, BMC Bioinf. 14 (2013) 128.
[20] M.V. Kuleshov, M.R. Jones, A.D. Rouillard, N.F. Fernandez, Q. Duan, Z. Wang, et al., Enrichr: a comprehensive gene set enrichment analysis web server 2016 update, Nucleic Acids Res. 44 (2016) W90–W97.
[21] S.T. Sherry, M.H. Ward, M. Kholodov, J. Baker, L. Phan, E.M. Smigielski, et al., dbSNP: the NCBI database of genetic variation, Nucleic Acids Res. 29 (2001) 308–311.
[22] L.D. Ward, M. Kellis HaploReg, A resource for exploring chromatin states, conservation, and regulatory motif alterations within sets of genetically linked variants, Nucleic Acids Res. 40 (2012) D930–D934.
[23] D.M. Evans, C.C. Spencer, J.J. Pointon, Z. Su, D. Harvey, G. Kochan, et al., Interaction between ERAP1 and HLA-B27 in ankylosing spondylitis implicates peptide handling in the mechanism for HLA-B27 in disease susceptibility, Nat. Genet. 43 (2011) 761–767.
[24] S. Saxonov, P. Berg, D.L. Brutlag, A genome-wide analysis of CpG dinucleotides in the human genome distinguishes two distinct classes of promoters, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 1412–1417.
[25] A.M. Deaton, A. Bird, CpG islands and the regulation of transcription, Genes Dev. 25 (2011) 1010–1022.
[26] S. Nolasco, J. Bellido, J. Goncalves, J.C. Zabala, H. Soares, Tubulin cofactor A gene silencing in mammalian cells induces changes in microtubule cytoskeleton, cell cycle arrest and cell death, FEBS Lett. 579 (2005) 3515–3524.
[27] Q. Gao, M.A. Frohman, Roles for the lipid-signaling enzyme MitoPLD in mitochondrial dynamics, piRNA biogenesis, and spermatogenesis, BMB Rep 45 (2012) 7–13.
[28] C. Vernet, M.-T. Ribouchon, G. Chimini, A.-M. Jouanolle, I. Sidibé, P.J.I. Pontarotti, A Novel Coding Sequence Belonging to a New Multicopy Gene Family Mapping within the Human MHC Class I Region vol. 38, (1993), pp. 47–53.
[29] J.K. Kulski, R.L. Dawkins, The P5 multicopy gene family in the MHC is related in sequence to human endogenous retroviruses HERV-L and HERV-16, Immunogenetics 49 (1999) 404–412.
[30] H. O'Geen, L. Echipare, P.J. Farnham, Using ChIP-seq technology to generate high-resolution profiles of histone modifications, Methods Mol. Biol. 791 (2011) 265–286.
[31] K.H. Yoo, L. Hennighausen, EZH2 methyltransferase and H3K27 methylation in breast cancer, Int. J. Biol. Sci. 8 (2012) 59–65.
[32] J.L. McClay, A.A. Shabalin, M.G. Dozmorov, D.E. Adkins, G. Kumar, S. Nerella, et al., High density methylation QTL analysis in human blood via next-generation sequencing of the methylated genomic DNA fraction, Genome Biol. 16 (2015) 291.
[33] N.E. Banovich, X. Lan, G. McVicker, B. van de Geijn, J.F. Degner, J.D. Blischak, et al., Methylation QTLs are associated with coordinated changes in transcription factor binding, histone modifications, and gene expression levels, PLoS Genet. 10 (2014) e1004663.
[34] Z. Li, K. Haynes, D.J. Pennisi, L.K. Anderson, X. Song, G.P. Thomas, et al., Epigenetic and gene expression analysis of ankylosing spondylitis-associated loci implicate immune cells and the gut in the disease pathogenesis, Genes Immun. 18 (2017) 135–143.