Epigenome-Wide Association Analysis Identified Nine Skin DNA Methylation Loci for Psoriasis

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Psoriasis is a chronic hyperproliferative and inflammatory skin disease caused by the interplay of genetic and environmental factors. DNA methylation has been linked to psoriasis, but the manner in which this process contributes to the disease is not fully understood. In this study, we carried out a three-stage epigenome-wide association study to identify disease-associated differentially methylated sites using a combination of 262 skin and 48 peripheral blood mononuclear cell samples. We not only revealed genome-wide methylation patterns for psoriasis but also identified strong associations between the skin-specific DNA methylation of nine disease-associated differentially methylated sites and psoriasis (Wilcoxon ranked $P_{\text{Bonferroni}} < 0.01$; methylation level difference > 0.10). Further analysis revealed that these nine disease-associated differentially methylated sites were not significantly affected by genetic variations, supporting their remarkable contributions to disease status. The expression of *CYP2S1*, *ECE1*, *EIF2C2*, *MAN1C1*, and *DLGAP4* was negatively correlated with DNA methylation. These findings will help us to better understand the molecular mechanism of psoriasis.

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INTRODUCTION

Psoriasis is a chronic immune-mediated skin disease that affects 0.17–4% of the population worldwide depending on the geographical location and ethnic diversity (Gelfand et al., 2005a, b; Shao et al., 1987). The etiology of psoriasis is incompletely understood, but evidence clearly suggests that epigenetics, specifically the methylation of cytosine residue at CpG dinucleotides, contributes to its development (Roberson et al., 2012). Although genomic DNA methylation (DNAm) has been studied in psoriasis and several other diseases, these studies were mainly based on the low-density Illumina HumanMethylation27 bead arrays, which focus on CpG loci mapping to promoter regions or CpG islands (CGIs) (Gervin et al., 2012; Roberson et al., 2012; Selamat et al., 2012). However, most of the reported disease-associated DNAm differences have occurred outside of these regions, emphasizing the necessity for examining DNAm profiles in nonpromoter and non-CGI regions to reveal insights into the pathogenesis of psoriasis (Busche et al., 2013; Irizarry et al., 2009).

DNAm is a reversible and heritable epigenetic process that participates in the transcriptional regulation and the control of both of alternative promoter usage and alternative splicing (Eckhardt et al., 2006; Maunakea et al., 2013). Several studies have shown that both genetic variations and environmental factors affect the DNAm patterns (Feil and Fraga, 2011; Lienert et al., 2011).

We conducted a genome-wide methylation study of psoriasis on 114 involved psoriatic (PP), 41 uninvolved psoriatic (PN), and 62 normal (NN) skin biopsies using a higher density Infinium Human-Methylation450 array (Illumina, San Diego, CA). This chip interrogates approximately 485,000 genomic loci at regions including 99% RefSeq genes and 96% CGIs, as well as CpG shores, noncoding RNAs and DNase-hypersensitive sites (Bibikova et al., 2011). We further evaluated the potential consequences arising from DNA methylation differences in target genes by performing transcriptome sequencing on 60 of the biopsied skin samples

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Abbreviations: CGI, CpG island; DMS, differentially methylated site; DNAm, DNA methylation; SNPs, single nucleotide polymorphisms

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(20 paired PP/PN and 20 NN) from the methylation panel. Supplementary Table S1 online summarizes the patients' clinical information, including sex, age, body mass index, and psoriasis area and severity index scores.

RESULTS

The global methylation pattern in normal skin tissues

Genome-scale methylation profiles were examined for 41 paired PP/PN, 73 PP, and 62 NN. After the filtering of probes that were ambiguously mapped and overlapped with single nucleotide polymorphisms (SNPs), we successfully detected 345,753 sites in all samples. We identified a bimodal distribution across the 62 NN samples, in addition to CpG sites, of which 33.6% were hypomethylated ($\beta < 0.2$, 0.08 [mean] ± 0.04 [standard deviation]; β is defined as the ratio of the signal intensity for the methylated allele over the sum of the methylated and unmethylated signal intensities) and 26.2% were hypermethylated ($\beta > 0.8$, 0.87 ± 0.04, Supplementary Figure S1 online). As expected, the DNAm level was strongly correlated with both the genomic location and the previously annotated regulatory regions (Supplementary Figures S2 and S3a and b online). Among the interrogated sites, 105,786 (30.9%) were located in CGIs. The mean methylation level was significantly lower for the CGI sites than for the non-CGI sites (0.23 ± 0.28 vs. 0.62 ± 0.28, $P < 1.0 \times 10^{-16}$, Supplementary Figure S3b). DNase I hypersensitive sites are regions with easily accessible chromatin that are functionally associated with gene transcriptional activity. From the Illumina annotation file, we found 43,264 sites that were located in DNase I hypersensitive sites, with a mean methylation level that was significantly lower than that of the non-DNase I hypersensitive site region (0.28 ± 0.25 and 0.53 ± 0.33, respectively, $P < 1.0 \times 10^{-16}$, Supplementary Figure S3c).

More than 90% of the CpG sites were stably methylated in the control samples, with a standard variation of less than 0.05. When the data were restricted to the top 10% most variable sites, we found that the intergenic (chi-squared $P < 2.2 \times 10^{-16}$) and CpG shore regions were enriched ($P = 2.7 \times 10^{-6}$), whereas the promoter and CGI regions were depleted (both $P < 2.2 \times 10^{-16}$, Figure 1a and b). The proportions of CpG probes varied across the categories, ranging from 0.3% for the 3′-untranslated regions to 26.3% for the CGIs in gene regions (Figure 1c and d). The mean standard variation also varied across the categories, ranging from 0.018 for the CGIs in ambiguous regions to 0.04 for the N-shores in nongene regions (Figure 1e and f).

Identification of differentially methylated sites in psoriasis

To identify sites with differential methylation in psoriasis, we performed stage I epigenome-wide association analysis between 41 paired PP and PN samples. At a Bonferroni-corrected $P_{\text{Bonferroni}} < 0.05$ and a minimum intergroup methylation difference of 0.1, we detected 1,514 differentially methylated sites (DMSs) by paired locus-by-locus Wilcoxon signed-ranked analysis. In the PP samples, 867 of these DMSs were significantly hypermethylated, whereas 647 were hypomethylated (Supplementary Data 1 and Supplementary Figure S4 online). The most significant DMS was located in the body of *SYTL3* (cg03003434, delta = −0.22, $P_{\text{Bonferroni}} = 3.14 \times 10^{-7}$), which is required for vesicular trafficking (Fukuda and Mikoshiba, 2001). Some of the most highly differentially methylated loci included epidermal differentiation complex genes: cg02331910 (*S100A13*, delta = 0.11, $P_{\text{Bonferroni}} = 2.47 \times 10^{-4}$), cg18348690 (*S100A10*, delta = 0.11, $P_{\text{Bonferroni}} = 2.08 \times 10^{-3}$), and cg08823182 (*S100A5*, delta = 0.11, $P_{\text{Bonferroni}} = 2.38 \times 10^{-3}$) were hypermethylated, whereas cg03165378 (*S100A9*, delta = −0.12, $P_{\text{Bonferroni}} = 1.57 \times 10^{-6}$), cg01431057 (*S100A8*, delta = −0.11, $P_{\text{Bonferroni}} = 7.86 \times 10^{-6}$), and cg17496887 (*S100A7A*, delta = −0.15, $P_{\text{Bonferroni}} = 1.73 \times 10^{-5}$) were hypomethylated (Supplementary Table S2 online). With the exception of cg01431057, which was located within the gene body, and cg16139316, which was located within the 5′-untranslated region, these epidermal differentiation complex sites were all located 1,500 bp upstream of the transcription sites (TSS1500). Six different *S100A* family genes passed the significance threshold, supporting the previous notion that skin structural proteins are of crucial importance in psoriasis (Kypriotou et al., 2012).

We next performed stage II epigenome-wide association analysis between the unrelated 73 PP and 62 NN samples and identified 426 DMSs using the unpaired locus-by-locus Wilcoxon rank-sum test at a $P_{\text{Bonferroni}} < 0.05$ (Supplementary Data 2 and Supplementary Figure S5 online). The most significant DMS was cg05590156, which was located in the body of *TRIO* (delta = 0.14, $P_{\text{Bonferroni}} = 4.24 \times 10^{-12}$). Among the remaining top ranked DMSs were two potential candidate psoriasis genes (Bak and Mikkelsen, 2010; Smith et al., 2003): cg19430423 (*CYP2S1*, delta = −0.16, $P_{\text{Bonferroni}} = 6.71 \times 10^{-12}$) and cg00288598 (*EIF2C2*, delta = −0.18, $P_{\text{Bonferroni}} = 5.40 \times 10^{-10}$, Supplementary Table S3 online). A total of 264 sites, of which 199 were located in or near 176 unique genes and 65 were intergenic, overlapped with the findings from stage I (Figure 2a). Among these 264 common DMSs, 16.7% (145 of 867) of the hypermethylated and 18.4% (119 of 647) of the hypomethylated DMSs found in stage I analysis were also detected in stage II analysis. Using the same significance threshold, we did not observe any DMSs between the 41 PN and 62 NN samples, indicating that these two nonpsoriatic skin types have similar methylation patterns (Figure 2b).

Validation of the methylation data

To further confirm our findings, we used a Sequenom Epityper system to validate the top 10% common DMSs (26 sites) and an additional three sites with an absolute delta > 0.15 from stage II analysis in a third independent cohort of 21 PP and 24 NN skin samples (Supplementary Table S4 online). Among these sites, 16 were successfully investigated, whereas analysis of the remainder failed because of technical limitations (Supplementary Methods online). Even with validation in a relatively smaller sample size, most of the sites feature a wider data range than those observed with an Illumina array platform (Supplementary Table S5 online). The Wilcoxon signed-ranked test revealed that nine CpG sites reached the significance threshold at a Bonferroni-adjusted *P*-value of 0.05 (Table 1). The most significant locus was located at the 5′-untranslated regions of *S100A8* (cg20335425, delta = −0.07, $P_{\text{Bonferroni}} = 2.06 \times 10^{-4}$). The