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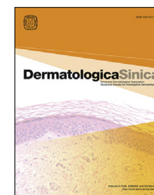

ORIGINAL ARTICLE

A lack of association between genetic polymorphisms in beta-defensins and susceptibility of psoriasis in Taiwanese: A case–control study

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ABSTRACT

Background: Genetic predisposition of the inflammatory-host response may affect the development of psoriasis. Previous studies have shown that copy number variations (CNVs) of β-defensin genes (*DEFB*) are associated with the susceptibility of psoriasis in Caucasian populations.
Objectives: This study aimed to assess the role of the CNVs of the *DEFB4* gene and functional variants in the *DEFB1* gene in Taiwanese patients with psoriasis.
Methods: In total, 196 patients with psoriasis and 196 control individuals were analyzed for the presence of the *DEFB4* CNVs using the paralogue ratio test, and also for the *DEFB1* polymorphisms rs11362, rs1800972, and rs1799946, using a polymerase chain reaction.
Results: None of the polymorphisms were found to be associated with psoriasis. The distribution of *DEFB4* genomic CNVs did not significantly differ between the control group and psoriasis group. The frequencies of patients who carried a greater than the median (≥ 5) number of copies did not significantly differ in patients with psoriasis and controls. The multivariate analysis similarly revealed that the *DEFB4* CNVs were not associated with psoriasis (odds ratio = 1.03, 95% confidence interval = 0.89–1.19, $p = 0.720$). No significant difference was detected in the genotype and allele distribution for any of the individual *DEFB1* polymorphisms between the cases and the controls. Finally, the overall haplotype frequency profiles derived from the three polymorphisms did not significantly differ between the cases and the controls.
Conclusion: Our results do not suggest that these genetic variants of the β-defensin genes contribute to the genetic background of psoriasis in Taiwanese patients.

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Introduction

Psoriatic skin lesions are characterized by histological evidence of inflammation, abnormal keratinocyte proliferation/terminal differentiation, and dermal angiogenesis.[1] Although the etiology of psoriasis remains unknown, it is clear that an interaction among genetic susceptibility variants, the immune system, and environmental factors contribute to the development of the chronic inflammatory process.[2]

Human β-defensins (hBDs) are a family of small, secreted antimicrobial peptides. In addition to antibacterial and antiviral effects,

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β-defensins have been shown to be involved in the immunological reactions that protect the host from various pathogens.[3] The expression of hBD-1 is generally constitutive, and the level of hBD-2 is thought to be induced by proinflammatory cytokines and bacteria.[4] Histologically, hBDs are expressed by epithelial cells of the skin, gut, respiratory tissue, and urogenital tissue.[5,6] In addition to epithelial cells, the expression of hBD-1 and hBD-2 have also been found in human monocytes, macrophages, and dendritic cells (DCs).[7]

HBDs are encoded by *DEFB* genes in three main gene clusters: two on chromosome 20 and one on chromosome 8p23.1. Of the eight β-defensin genes at 8p23.1, not including *DEFB1* (encoding the protein hBD-1) but including *DEFB4* (encoding the protein hBD-2), *SPAG11*, *DEFB103* (encoding the protein hBD-3), *DEFB104* (encoding the protein hBD-4), *DEFB105*, *DEFB106*, and *DEFB107*, are on a large repeat unit that varies in copy number. In humans, up to 12 copies of this repeat have been found, and three to five copies per diploid genome are more prevalent.[8] HBD-2, hBD-3, and hBD-4 have been demonstrated to stimulate keratinocytes to release interleukin (IL) IL-8, IL-18, and IL-20, which are all proinflammatory cytokines that have an established role in the pathogenesis of psoriasis.[9] Recently, Hollox et al[10] found an association between higher copy number variations (CNVs) for *DEFBs* on chromosome 8p23.1 and risk of psoriasis in a Caucasian population. However, the relationship of *DEFBs* CNVs and psoriasis, until now, remains unclear in the Chinese population. Further, the role of the *DEFB1* gene as a potential modifier in psoriasis has not previously been studied. Three singe nucleotide polymorphisms (SNPs) at positions c.-20G>A (rs11362), c.-44C>G (rs1800972), and c.-52G>A (rs1799946) in the 5'-untranslated region of *DEFB1* gene have been described to influence the hBD-1 expression or function.[11,12] Recent studies in the Mexican, Egyptian, and Korean populations have identified an association between *DEFB1* SNPs and the susceptibility of atopic dermatitis,[13–15] which shares with psoriasis the similar phenotypes of dry, scaly skin and disturbed epidermal differentiation. We, therefore, consider it to be important to investigate the relevance of the SNPs of the *DEFB1* gene and the CNVs of the *DEB4* genes in patients with psoriasis among the Taiwanese population.

Methods

Study population

One hundred and ninety-six patients with chronic plaque psoriasis and 196 control participants, comparable for age and gender, were enrolled in this study. Patients with psoriasis were diagnosed by means of clinical and/or histopathological findings. Severity of psoriasis was categorized into three divisions based on the area on the body affected by psoriasis: (1) affected surface of < 5% was categorized as mild psoriasis; (2) 5–10% affected area was moderate psoriasis; and (3) > 10% affected area was severe psoriasis. Patients whose disease onset was younger than 40 years were considered to have "early onset" psoriasis, and those whose disease onset was older than 40 years were diagnosed as "late onset". Control participants responded to a questionnaire on their medical history and lifestyle characteristics, and they were recruited during routine health examinations from which no clinical evidence of psoriasis was found by the research nurses. The study protocol was approved by the hospital's ethics committee (Chang Gung Medical Foundation Institutional Review Board (Taipei, Taiwan; 100-4528A3 and 103-7245C), and conducted in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from all participants.

Genomic DNA extraction

Genomic DNA of patients and controls was respectively isolated from oral epithelial cells and peripheral blood leukocytes, with the standard method using proteinase K digestion of the nuclei.

Paralogue ratio test-based restriction enzyme digest variant ratio

A paralogue ratio test (PRT) was performed, as previously reported,[16] using the HSPDP3 system (a heat-shock protein pseudogene of ~2 kb), which specifically amplified chromosome 8 (*DEFB4*) in conjunction with reference products from chromosome 5, with products differing by 4 bp. The primers were HSPD5.8F (CCAGATGAGACCAGTGTCC) and FAM-labelled HSPD5.8R (TTTTAAGTTCAGCAATTACAGC). Genomic DNA of 5 ng was amplified in a final volume of 20 mL, with 0.5mM forward primer and 0.5mM FAM-labelled reverse primer, in a buffer containing final concentrations of 50mM Tris–*hydrochloride* (pH 8.8), 12mM ammonium sulfate, 5mM magnesium chloride, 125 mg/mL *bovine serum albumin* (non-acetylated, Ambion Inc., Austin, TX, USA), 7.4mM 2-mercaptoethanol, and 1.1mM each deoxynucleotide (sodium salts), with 0.5U Taq DNA polymerase in a total volume of 10 μL. Products were amplified using 30 cycles of 95°C for 30 seconds, 53°C for 30 seconds, and 70°C for 30 seconds, followed by a single "chase" phase of 53°C for 1 min/70°C for 20 minutes. One μL of each polymerase chain reaction product was added to a 10 μL digestion containing 1× ReAct 2 buffer [50mM Tris-hydrochloride (pH 8.0), 10mM MgCl2, and 50mM NaCl] (Invitrogen, CA, USA) and 5U *Hae*III (New England Biolabs, MA, USA). After incubation at 37°C for 4–16 hours, 2 μL was added to 10 μL HiDi formamide with ROX-500 marker (Applied Biosystems, Warrington, UK), and analyzed using electrophoresis on an ABI3100 (Applied Biosystems, Foster City, CA) 36 cm capillary using the POP4 polymer. The test (chromosome 8 *DEFB4*) and reference (chromosome 5) amplicons were distinguished by using the GeneScan Analysis software (PE Applied Biosystems, Foster City, CA).

Genotyping of DEFB1 -20G>A (rs11362), -44C>G (rs1800972), and -52G>A (rs1799946) polymorphisms

To determine the genotype of the rs11362 polymorphism, polymerase chain reaction amplification was performed using a forward primer 5′-CAGGGGTTAGCGATTAG-3′, and a reverse primer 5′-GCAGAGAGTAAACAGAAGGTA-3′. An amplified product of 227 bp was digested with *BstNI*, with resulting fragments of 167 bp for the GG wild type, 227 bp for the AA homozygote, and 167 bp and 227 bp for the GA heterozygote. Genotyping for the SNP rs1800972 and rs1799946 were performed using TaqMan SNP Genotyping Assays obtained from Applied Biosystems (ABI, Foster City, CA, USA).

Statistical analyses

The clinical characteristics of continuous variables were expressed as mean ± standard deviation, and were tested using a two-sample *t* test. Either the Chi-square test or Fisher's exact test was used to examine the differences in categorical variables and to compare the allele and genotype frequencies. The Kolmogorov–Smirnov test was used to compare the shape of the CNVs frequency distributions. Binary logistic regression analysis was used to evaluate the independent effect of investigated genotypes on the risk of psoriasis, adjusted for age, gender, smoking, diabetes, hypertension, and body mass index (BMI). The analysis of deviation from the Hardy–Weinberg equilibrium, estimation of the linkage disequilibrium

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