

## ORIGINAL ARTICLE

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



Yu-Jui Hsieh<sup>1, a</sup>, Ya-Ching Chang<sup>2, \*, a</sup>, Yu-Huei Huang<sup>2</sup>, Wen-Hung Chung<sup>2</sup>,  
Hsin-Yi Tsai<sup>3</sup>, Lung-An Hsu<sup>3</sup>

<sup>1</sup> Department of Medicine, Chang Gung University College of Medicine, Tao-Yuan, Taiwan

<sup>2</sup> Department of Dermatology, Chang Gung Memorial Hospital and Chang Gung University College of Medicine, Taipei, Taiwan

<sup>3</sup> First Cardiovascular Division, Department of Internal Medicine, Chang Gung Memorial Hospital and Chang Gung University College of Medicine, Tao-Yuan, Taiwan

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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  $\beta$ -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 ( $\geq 5$ ) 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  $\beta$ -defensin genes contribute to the genetic background of psoriasis in Taiwanese patients.

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\* Corresponding author. Department of Dermatology, Chang Gung Memorial Hospital and Chang Gung University College of Medicine, Taipei, Taiwan, Number 199, Tung-Hwa North Road, Taipei 105, Taiwan.

E-mail address: [ycchang@adm.cgmh.org.tw](mailto:ycchang@adm.cgmh.org.tw) (Y.-C. Chang).

<sup>a</sup> These authors contribute equally to this work.

## Introduction

Psoriatic skin lesions are characterized by histological evidence of inflammation, abnormal keratinocyte proliferation/terminal differentiation, and dermal angiogenesis.<sup>1</sup> 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.<sup>2</sup>

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

$\beta$ -defensins have been shown to be involved in the immunological reactions that protect the host from various pathogens.<sup>3</sup> The expression of hBD-1 is generally constitutive, and the level of hBD-2 is thought to be induced by proinflammatory cytokines and bacteria.<sup>4</sup> Histologically, hBDs are expressed by epithelial cells of the skin, gut, respiratory tissue, and urogenital tissue.<sup>5,6</sup> 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).<sup>7</sup>

HBDs are encoded by *DEFB* genes in three main gene clusters: two on chromosome 20 and one on chromosome 8p23.1. Of the eight  $\beta$ -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.<sup>8</sup> 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.<sup>9</sup> Recently, Hollox et al.<sup>10</sup> 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 single 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.<sup>11,12</sup> Recent studies in the Mexican, Egyptian, and Korean populations have identified an association between *DEFB1* SNPs and the susceptibility of atopic dermatitis,<sup>13–15</sup> 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,<sup>16</sup> 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 (CCA-GATGAGACCAGTGTCC) and FAM-labelled HSPD5.8R (TTTAAAGTT-CAGCAATTACAGC). Genomic DNA of 5 ng was amplified in a final volume of 20  $\mu$ L, 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  $\mu$ L. 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  $\mu$ L of each polymerase chain reaction product was added to a 10  $\mu$ L digestion containing 1  $\times$  ReAct 2 buffer [50mM Tris-hydrochloride (pH 8.0), 10mM MgCl<sub>2</sub>, 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  $\mu$ L was added to 10  $\mu$ L 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 *Bst*NI, 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  $\pm$  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

between the polymorphisms, and haplotype analysis were performed using SNPStats software (available at <http://bioinfo.iconcologia.net/SNPstats>).

## Results

**Table 1** lists the characteristics of the cases and control participants. There was no significant difference between the two groups with regard to gender, age, hypertension, diabetes, smoking, and BMI. The distributions of the *DEFB4* genomic copy number in the study population, shown in **Figure 1**, exhibited a range of 2–11 per genome, with a median number of five copies. The difference in overall *DEFB4* genomic copy number distribution between psoriasis patients and control participants was insignificant, as determined with the Kolmogorov–Smirnov test ( $p = 0.657$ ). The proportions of control individuals who carried a number of copies less than the median ( $< 5$ ) or greater than median ( $\geq 5$ ) were 42.9% and 57.1%, respectively. In the patients with psoriasis, the frequency of distribution of the subgroup (38.8% and 61.2%) did not significantly differ from that of the control group ( $p = 0.411$ ; **Figure 2**). Individuals with a copy number  $\geq 5$  had an insignificantly higher risk of psoriasis than did individuals with a copy number  $< 5$  (odds ratio = 1.18, 95% confidence interval = 0.79–1.77). The average number of copies per genome in psoriasis patients was slightly greater than that of the control participants ( $5.14 \pm 2.01$  vs.  $5.02 \pm 1.75$ ), but the difference did not reach statistical significance ( $p = 0.520$ ). In the logistic regression analysis with the *DEFB4* CNVs as an integer variable, they were also not associated with psoriasis after adjusting for age, sex, smoking, diabetes, hypertension, and BMI (odds ratio = 1.03, 95% confidence interval = 0.89–1.19,  $p = 0.720$ ). Additionally, comparison between the disease subgroup (i.e., early/late onset and severity of psoriasis) among psoriasis patients, revealed no significant difference in the frequencies of the carriers with a copy number  $\geq 5$  (data not shown).

The genotype and allele frequencies of the *DFEB1* –20G>A (rs11362), –44C>G (rs1800972), and –52G>A (rs1799946) polymorphisms in psoriasis patients and controls are presented in **Table 2**. Some of the study participants could not be genotyped for the polymorphisms because their DNA samples had been used up for other studies. No significant deviation from the Hardy–Weinberg equilibrium was detected for the *DFEB1* SNPs in either the study cases or the controls. All SNPs were in strong pairwise linkage disequilibrium. As can be seen in **Table 2**, none of the polymorphisms were found to be associated with psoriasis. No significant difference was observed in genotype and allele distribution for any of the *DEFB1* polymorphisms between the cases and the controls. After adjustment for age, sex, smoking, diabetes, hypertension, and BMI, the results remained unchanged (data not shown). Additionally, the overall haplotype frequency profiles did not differ significantly between the psoriasis cases and the controls (**Table 3**;  $p = 0.77$ ). Furthermore, comparison between the disease subgroup

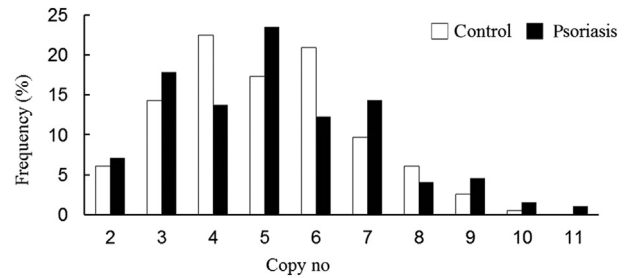
**Table 1** Clinical characteristics of the psoriatic patients and controls.

	Controls	Patients	<i>p</i>
No.	196	196	
Age (y)	48 ± 11	49 ± 16	0.295 <sup>a</sup>
Men/women	152/44	154/42	0.807 <sup>b</sup>
Smoking (%)	32.7	25.0	0.094 <sup>b</sup>
Diabetes mellitus (%)	3.1	7.1	0.066 <sup>b</sup>
Hypertension (%)	15.82	19.4	0.353 <sup>b</sup>
Body mass index (kg/m <sup>2</sup> ) <sup>c</sup>	25.2 ± 3.4	25.3 ± 4.7	0.821 <sup>a</sup>

<sup>a</sup> By two-sample *t* test

<sup>b</sup> By  $\chi^2$  test

<sup>c</sup> Ninety-eight psoriasis patients had missing data on body mass index status.



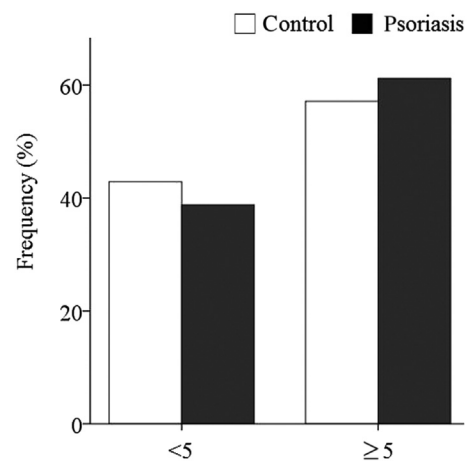
**Figure 1** Frequency distribution of the *DEFB4* copy numbers in controls ( $n = 196$ ) and psoriasis patients ( $n = 196$ ).

(i.e., early/late onset and severity of psoriasis) among psoriasis patients, revealed no significant difference in the frequencies of the *DEFB1* alleles and genotypes (data not shown).

## Discussion

In the present case–control study, we found no evidence of an association between SNPs rs11362, rs1800972, and rs1799946 in the *DEFB1* gene and the risk of psoriasis. There were also no relationships between the *DEFB4* CNVs and the risk of psoriasis. Our results therefore suggest that these polymorphisms are not important risk factors for psoriasis among Chinese living in Taiwan.

$\beta$ -defensins are antimicrobial peptides that are also proinflammatory because they act as chemokines for immune cells, such as T cells and DCs.<sup>17</sup> Hollox et al.<sup>10</sup> first reported the association between the risk of psoriasis and increased  $\beta$ -defensin genomic copy number. Individuals with a copy number  $> 5$  are about five times more likely to have psoriasis than individuals with a copy number of 2, and there is a significant linear increase in relative risk with increasing copy number (odds ratio around 1.32). This association was replicated in a subsequent study with three cohorts of European origin, although with a reduced odds ratio of 1.081.<sup>18</sup> A previous study showed a positive relationship between  $\beta$ -defensin copy number and mucosal hBD-2 mRNA expression in patients with colon Crohn disease.<sup>19</sup> Recently, Jansen et al.<sup>20</sup> have further demonstrated a significant correlation between serum hBD-2 levels and  $\beta$ -defensin copy number in healthy individuals. Moreover, hBD-2 is strongly expressed in lesional psoriatic epidermis and high systemic hBD-2 levels are strongly correlated with disease



**Figure 2** Distribution of the *DEFB4* copy numbers in controls (open bars) and psoriasis patients (gray bars) allocated to  $< 5$ , or  $\geq 5$  copies per genome. The *p* value = 0.411 (using  $\chi^2$  test) for the controls versus psoriatic patients.

**Table 2** Allele and genotype frequencies of the single nucleotide polymorphisms rs11362, rs1800972, and rs1799946 in the *DEFB1* gene in the study population.

	Genotype	Genotype		$p^a$	Allele		$p^a$
rs11362	GG	GA	AA	0.609	G	A	0.818
Controls (n = 184)	72 (39.1)	82 (44.6)	30 (16.3)		0.614	0.386	
Psoriasis (n = 188)	71 (37.8)	92 (48.9)	25 (13.3)	0.622	0.378		
rs1800972	CC	CG	GG	0.061	C	G	0.784
Controls (n = 187)	147 (78.6)	36 (19.3)	4 (2.1)		0.882	0.118	
Psoriasis (n = 171)	133 (77.8)	38 (22.2)	0 (0)	0.889	0.111		
rs1799946	GG	GA	AA	0.075	G	A	0.772
Controls (n = 190)	54 (28.4)	83 (43.7)	53 (27.9)		0.503	0.497	
Psoriasis (n = 190)	41 (21.6)	105 (55.3)	44 (23.2)	0.492	0.508		

Data are presented as n (%).

<sup>a</sup> Two-sided  $\chi^2$  test for distributions of either genotype or allele frequencies between the cases and controls.

activity as assessed by the psoriasis area and severity index score.<sup>20</sup> Therefore, it is hypothesized that an inappropriately high amount of hBD-2 leads to an inappropriate inflammatory response, thereby triggering a psoriatic plaque. However, we could not replicate this association in a Chinese population living in Taiwan, although the average number of copies per genome in psoriasis patients was slightly greater than that of control participants. The differences may be due to ethnic variation in diplotype frequency distributions or environmental influences. Accurate copy number typing of the  $\beta$ -defensin CNV region has been challenging, and only the PRT method was used in this study. However, the PRT method has been shown to provide a high degree of concordance with other methods, including multiplex amplifiable probe hybridization, multiple ligation probe amplification, and array-comparative genomic hybridization.<sup>16</sup> Our data, which accords with previous findings, revealed that the distributions of Chinese populations shifted slightly toward higher copy numbers compared with European populations.<sup>21</sup> Another possible reason to explain the apparent genetic difference of our findings is the small sample size for our study. A sample size of nearly 10,000 cases and controls is required to detect such a small genetic effect with 80% statistical power.

To the best of our knowledge, this is the first investigation regarding the effect of the *DEFB1* polymorphisms on the risk of psoriasis. Previous studies have found an association between the *DEFB1* SNPs and the susceptibility of atopic dermatitis in different ethnic populations.<sup>13–15</sup> It has been reported that the rs11362, rs1800972, and rs1799946 in the 5'- untranslated region of the *DEFB1* gene may cause functional variations in the expression of hBD-1<sup>11,12</sup> and are associated with autoimmune diseases like systemic lupus erythematosus.<sup>22</sup> However, our study was unable to demonstrate an association between psoriasis and genetic variants of *DEFB1*. HBD-1 mRNA and peptide were found to be expressed consistently in the skin samples from various body sites and localized to the suprabasal keratinocytes, sweat ducts, and sebaceous glands of human skin.<sup>23</sup> Both hBD-1 and hBD-2 exhibit chemotactic activity for cells stably transfected with CC chemokine receptor 6 (CCR6). HBD-1 may function through interaction with

CCR6 to recruit immature DCs and memory T cells to cutaneous sites of microbial invasion.<sup>17</sup> CCR6 was found to be required for IL-23-induced psoriasis-like inflammation in mice,<sup>24</sup> and is considered to be a therapeutic target in psoriasis.<sup>25,26</sup> However, gene expression of hBD-1 in keratinocytes, in contrast to hBD-2, is not subject to regulation by proinflammatory cytokines,<sup>27</sup> as it is not highly induced in psoriatic skin.<sup>20</sup> In addition, enhanced proinflammatory cytokines expression by keratinocytes is associated with the stimulation by hBD-2 but not hBD-1.<sup>9</sup> Taken together, our research suggests that *DEFB1* may not play an important role in the development of psoriasis.

Our study has several limitations. Firstly, because the sample size of our study is relatively small, we cannot on the basis of our results exclude the possibility of a relatively small risk of psoriasis associated with the studied CNVs and SNPs. Secondly, we did not assess the effect of the *DEFB1* variants on the local hBD-1 and hBD-2 expressions in psoriatic plaques. Moreover, our study has a cross-sectional design and provides no information about the effect of the *DEFB1* variants on the progression of psoriasis or its clinical outcome. A larger prospective and longitudinal study would be necessary to fully assess the significance of these polymorphisms in psoriasis. Moreover, the examined patients were ethnically Chinese, and caution should be exercised when extrapolating our results to other ethnic groups.

In conclusion, our study results do not support the associations of susceptibility with psoriasis among Taiwanese with the *DEFB1* -20G>A, -44C>G, -52G>A SNPs, and *DEFB4* CNVs.

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**Table 3** Frequencies of the common haplotypes derived from the single nucleotide polymorphisms rs11362, rs1800972, and rs1799946 in the *DEFB1* gene in the study population.

	rs11362	rs1800972	rs1799946	Total*	Controls	Psoriasis
H1 <sup>a</sup>	G	C	A	0.4883	0.4784	0.4989
H2	A	C	G	0.371	0.3741	0.3679
H3	G	G	G	0.108	0.1108	0.1050
H4	G	C	G	0.0185	0.0172	0.0198
H5	A	C	A	0.0097	0.0110	0.0084

\*  $p = 0.77$ .

<sup>a</sup> Likelihood ratio test for global haplotype effect.

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