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ORIGINAL RESEARCH

Psoriasis Patients with Specific HLA-Cw Alleles and Lower Plasma IL-17 Level Show Improved Response to Topical Lindioil Treatment

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Purpose: Lindioil, a medicine refined from indigo naturalis (a herb used in Chinese medicine), is effective in treating severe psoriasis; however, responses vary across individual patients. We aim to investigate genetic predispositions associated with treatment response to topical Lindioil among patients with psoriasis and correlations with plasma cytokine patterns.

Patients and Methods: We enrolled 72 psoriasis patients treated with Lindioil ointment and analyzed the human leukocyte antigen class C (*HLA-Cw*) genotypes and plasma cytokine expression patterns. We developed regression models of treatment response, defined as Psoriasis Area and Severity Index (PASI) 75, to examine correlations among HLA-Cw alleles, cytokine levels, and treatment response to Lindioil.

Results: Patients harboring *HLA-Cw*06:02* were significantly more likely to respond to Lindioil ($P = 0.02$, odds ratio [OR]: 6.88), whereas Lindoil was ineffective in those harboring *HLA-Cw*01:02* ($P = 0.01$, OR: 0.28). Patients who were *HLA-Cw*06:02*-positive or *HLA-Cw*01:02*-negative had better PASI scores and body surface area (BSA) improvement (73.3% vs 44.4%, $P<0.001$) following an 8-week treatment period. Psoriasis patients achieving PASI 75 after 8 weeks presented with lower baseline plasma interleukin-17 (IL-17) levels than those who did not achieve PASI 75 (PASI 75: 11.28 pg/mL vs PASI <75: 15.82 pg/mL, $P = 0.05$).

Conclusion: Our findings suggest that the presence of the *HLA-Cw*06:02* or *HLA-Cw*01:02* alleles and plasma IL-17 levels are predictive markers of treatment response to Lindioil ointment in patients with psoriasis.

Keywords: *HLA-Cw*06:02*, *HLA-Cw*01:02*, indigo naturalis, Lindioil, IL-17, psoriasis

Introduction

Psoriasis is a common, chronic, immune-mediated, inflammatory skin disease. Psoriasis results from an aberrant innate or adaptive immune response associated with T lymphocytes that leads to epidermal hyperplasia[1] and is characterized by salmon-pink plaques with heavy silver scales.[2] Psoriasis has negative effects on the patient's health and quality of life, including psychosocial and economic impacts.[3,4] The disease pathogenesis represents a complex interplay among genetic, environmental, and immunological factors. Certain alleles encoding human leukocyte antigen-Cw (HLA-Cw), such as *HLA-Cw*06:02*, have been identified as important genetic determinants of psoriasis development.[5] HLA-Cw gene expression has been linked to psoriasis incidence, severity, phenotypic features, and treatment outcomes.[6] The

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presence of *HLA-Cw*06:02* has been suggested to serve as a predictive marker for the clinical response to ustekinumab (IL-12/23 antibody) and methotrexate treatment in psoriasis.[7,8] Various therapies with different targets have been developed to alleviate psoriasis symptoms, including systemic therapy, phototherapy, topical therapy, and traditional Chinese medicine (TCM). The topical application of ointments developed from indigo naturalis a commonly used herb in TCM, has been proposed as an attractive treatment option with lower costs and reduced occurrence of long-term side effects compared with other anti-psoriasis treatments.[9,10]

Indigo naturalis is derived from a blue powder extracted from indigo plants, such as *Baphicacanthus cusia*.[11] Indigo naturalis has been widely used in TCM for the treatment of inflammatory diseases and dermatosis for centuries.[12] Indigo naturalis has been demonstrated to have anti-inflammatory and anti-proliferative properties through several mechanisms, mediated by its active components, including indirubin, indigo, and tryptanthrin.[13] Indirubin, the important primary active component, inhibits the proliferation of epidermal keratinocytes through the inhibition of cyclin-dependent kinase[14,15] and epidermal growth factor receptor (EGFR) activation.[16,17] In addition, indirubin suppresses skin inflammation by inhibiting cytokine production, nuclear factor kappa B (NF-κB) activity, and mitogen-activated protein kinase signaling.[18,19] Tryptanthrin also possesses anti-inflammatory properties that attenuate the T-helper 17 (Th17) signature[12] and downregulate the IL-17 pathway.[20] Another component, indigo, is less active than the other two,[10] but both indigo and indirubin activate the aryl hydrocarbon receptor signaling pathway to modulate the immune response.[21,22] Indigo naturalis in its crude form has also been shown to reduce T cell adhesion by suppressing tumor necrosis factor (TNF)-α–induced expression of vascular cell adhesion molecule 1 (VCAM-1).[23]

Lindioil is a refined form of indigo naturalis ointment powder, with the original blue color removed to prevent staining (staining of skin and clothes was the reason for poor patient compliance with crude indigo naturalis ointment); Lindoil remains a safe and effective treatment.[10] Our Clinical trial[11] showed that 200 μg/g of indirubin in Lindioil ointment was the most effective concentration for topical psoriasis treatment. However, treatment response varied across individual patients, and the underlying mechanisms that lead to differential responses remain unclear. Although the HLA-Cw allele and the IL-17/IL-23 mediated pathway are regarded as important factors in the response to psoriasis treatment, the associations among HLA allele status, plasma cytokine levels, and response to Lindioil treatment have not yet been thoroughly explored. Therefore, to improve our ability to provide precise and effective treatment options, we attempted to identify useful biomarkers for predicting the treatment response to Lindioil ointment among psoriasis patients.

Materials and Methods

Study Population

Between November 2012 and April 2014, 72 patients were identified for study inclusion who were diagnosed with plaque psoriasis for at least 1 year, with <20% of their body surface area (BSA) affected by psoriasis, and a Psoriasis Area and Severity Index (PASI) sCore <20. Washout periods of at least 4 weeks following the end of systemic therapy or phototherapy regimens and at least 2 weeks following the end of topical therapies were required prior to the initiation of study medication. All patients were previously enrolled in our clinical trial study, which is registered with ClinicalTrials.gov (identifier NCT01735864), and were treated with Lindioil for at least 8 weeks. After completion of the clinical trial, we used the remaining DNA and plasma samples from enrolled patients to perform HLA-Cw genotyping and evaluate cytokine expression, respectively. All participants provided written informed consent and agreed to the use of their samples for this study (IRB No. 201507317B0). The study was conducted at Chang Gung Memorial Hospital, Linkou, and Taipei branches. The protocol was approved by the institutional review boards of Chang Gung Memorial Hospital in Taiwan. The study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practices.

Human Leukocyte Antigen Genotyping

All 72 patients were genotyped for HLA-Cw alleles using SeCore HLA sequence–based typing (SBT) (Invitrogen, Life Technologies, Carlsbad, CA). The HLA-Cw genotyping data for 507 individuals in the general Chinese population with no history of severe skin disorders was obtained as described previously.[24]

Cytokine Level Analysis

The 64 patients with psoriasis were analyzed for cytokine expression patterns, including IL-6, IL-17, IL-22, IL-23, and TNF-α. The concentrations of these cytokines in plasma were determined by using the Bio-Plex pro assays (Bio-Rad Laboratories, Irvine, California), according to the manufacturer's instructions. All samples were analyzed in duplicate.

Definition of Treatment Response

PASI scores were recorded at baseline and weeks 8, 12, and 20 following initiation of treatment. The mean percentage of PASI score improvement at weeks 8, 12, and 20 were calculated, and the proportions of patients achieving at least 50% reduction (PASI 50), 75% reduction (PASI 75), and 90% reduction (PASI 90) at each time point were compared between HLA genotypes. Positive treatment response was defined as at least PASI 75 relative to baseline. Patients achieving PASI 75 were considered good responders, whereas those who did not achieve PASI 75 were classified as poor responders. We evaluated the mean percentage of improvement in PASI and BSA scores at weeks 8, 12, and 20.

Statistical Analyses

Statistical analysis was performed using SPSS for Windows software version 21.0 (IBM Corp, Armonk, NY). Data are presented as the number (percentage) for categorical variables and as the mean ± standard deviation (SD) for continuous variables. Differences were evaluated using Fisher's exact test for categorical variables, whereas an independent two-tailed *t*-test was used to evaluate differences in continuous variables. Only those variables with $P<0.05$ in univariate analyses were included in the multiple regression analysis for treatment response. A two-tailed *P*-value <0.05 was considered significant.

Results

Table 1 shows the demographic and clinical characteristics of the 72 patients with psoriasis (including 43 men and 29 women) treated with Lindioil included in this study. The mean age of the patients was 35.3±8.2 years, and the mean body mass index was 24.6±4.1 kg/m^2. The mean duration of psoriasis was 11.2±7.5 years, and all patients (100%) had received prior treatment, including 2 patients treated with the biologic etanercept. In addition, 98.6% used topical therapy, and 76.4% of patients used Chinese herbs in either oral or topical forms. The mean PASI score and BSA% affected by psoriasis at baseline were 11.8±4.2 and 10.6%±6.0%, respectively. The mean duration of Lindioil treatment was 17.0±5.6 weeks. After 8 weeks of treatment, 51.4% had achieved at least PASI 50, and 47.2% of patients had achieved PASI 75. However, 5 patients (6.9%) experienced treatment-related adverse events (AEs). Treatment-related AEs in our study included acute psoriasis flare-ups, contact dermatitis, allergic exanthema, eczema, and skin inflammation.

We first performed SBT to determine the individual HLA-Cw genotypes among our study population (Supplementary Table S1) and found that the *HLA-Cw*06:01* allele was significantly associated with psoriasis compared with 507 healthy controls with no history of severe skin disorders ($P=0.0008$, Supplementary Table S2). Further analysis of the correlations between HLA-Cw status and treatment response to Lindioil revealed that patients had poorer responses when carrying the *HLA-Cw*01:02* allele (good responders vs poor responders carrying the *HLA-Cw*01:02* allele, 29.4% vs 57.9%; odds ratio [OR]: 0.60, 95% confidence interval [CI], 0.39–0.92; $P=0.019$, Table 2), whereas those had better responses when carrying the *HLA-Cw*06:02* allele (good responders vs poor responders carrying the *HLA-Cw*06:02* allele, 29.4% vs 7.9%; OR: 1.31; 95% CI: 1.03–1.65; $P=0.03$, Table 2). In the multivariate analysis, *HLA-Cw*01:02* was associated with the treatment response (OR: 0.35; 95% CI" 0.13–0.96; $P=0.042$, Supplementary Table S3). No significant differences of demographic, clinical features and adverse event were observed between the good and poor responder groups (Table 2). To improve the sensitivity to predict the response to Lindioil treatment, we combined these two associated HLA-Cw alleles and found that 73.5% (25 in 34 patients) patients who achieved PASI 75 (good responders) at week 8 were *HLA-Cw*01:02*-negative or *HLA-Cw*06:02*-positive ($P=0.031$, Table 2).

We further analyzed and compared these two *HLA* genes after 20 weeks of Lindioil treatment. Among our cohort (Table 3 and Figure 1A–C), 32 patients (44.4%) were *HLA-Cw*01:02*-positive and 40 patients (55.6%) were *HLA-Cw*01:02*-negative. The *HLA-Cw*01:02*-negative group had a longer psoriasis duration than the *HLA-Cw*01:02*-positive group

Table 1 Demographic and Clinical Characteristics of Enrolled Study Population

Parameter	Number or Value
Number	72
Male, n (%)	43 (59.7)
Body Mass Index(kg/m^2), mean±SD	24.6±4.1
Mean Age(years), mean±SD	35.3±8.2
Age at onset of disease(years), mean±SD	24.1±9.3
Duration of psoriasis(years), mean±SD	11.2±7.5
Allele frequency of HLA-Cw*01:02, n (%)	32 (44.4)
Allele frequency of HLA-Cw*06:02, n (%)	13 (18.1)
Previous treatment for psoriasis, n (%)	72 (100)
Topical therapy	71 (98.6)
Phototherapy	17 (23.6)
Systemic nonbiological therapy	13 (18.1)
Systemic biological therapy	2 (2.8)[#]
Chinese herbs	55 (76.4)
Other	11 (15.3)
Baseline PASI score, mean±SD	11.8±4.2
Baseline BSA% affected by psoriasis, mean±SD	10.6±6.0
Duration of Lindioil therapy (weeks)	17.0±5.6
PASI 75 at week 8	34 (47.2)
PASI 75 at week 12	30 (41.7)
PASI 75 at week 20	31 (43.1)[@]
Adverse events	5 (6.9)

Notes: [#]Two of them received etanercept as biologic treatment; [@]the intermittent missing data were imputed by last-observation-carried-forward for efficacy data.
Abbreviations: PASI, Psoriasis Area and Severity Index; BSA, body surface area.

Table 2 Demographic and Clinical Characteristics of Good and Poor Responders Treated with Lindioil Ointment

Demographic Data	Good Lindioil Responders (n=34)	Poor Lindioil Responders (n=38)	*P* value*
Demographic characteristics			
Male	17 (50.0)[#]	26 (68.4)	0.150
Mean Age (years)	35.9±8.6[#]	34.8±8.0	0.582
Body Mass Index (kg/m^2)	24.0±3.8	25.1±4.4	0.279
Clinical characteristics			
Age at onset of disease (years)	24.6±8.9	23.7±9.9	0.677
Duration of psoriasis (years)	11.3±6.7	11.5±8.7	0.892
Previous treatment for psoriasis	34 (100)	38 (100)	1
Cumulative dose of Lindioil (g)	699.5±579.2	893.1±768.3	0.236
PASI score at baseline	12.0±4.2	11.6±4.4	0.664
BSA% at baseline	11.4±6.1	9.8±5.9	0.279
*Cw*0102*	10 (29.4)	22 (57.9)	**0.019**
*Cw*0304*	3 (8.8)	7 (18.4)	0.316
*Cw*0602*	10 (29.4)	3 (7.9)	**0.03**
*Cw*0702*	17 (50.0)	14 (36.8)	0.341
*Cw*01:02* NEG or *Cw*06:02* POS	25 (73.5)	18 (47.4)	**0.031**
Adverse event	2 (5.9)	3 (7.9)	1.000

Notes: [#]Data was presented as number (percentage) for discrete variables and mean ± standard deviation for continuous variables. **P* value was calculated by Fisher's exact test or independent two-tailed *t*-test. Bold text was presented to emphasize *P* value<0.05.
Abbreviations: PASI, psoriasis area severity index; BSA, body surface area; HLA, human leukocyte antigen.

Table 3 Demographic and Clinical Characteristics of *HLA-Cw*01:02* Positive/Negative Patients Treated with Lindioil

Demographic data	*Cw*01:02* Positive (n=32)	*Cw*01:02* Negative (n=40)	*P* value*
Demographic characteristics			
Male, n (%)	20 (62.5)	23 (57.5)	0.810
Mean Age (years), mean±SD	35.0±9.0	35.6±7.7	0.785
Body mass index (kg/m^2), mean±SD	23.8±4.2	25.3±4.0	0.123
Clinical characteristics			
Age at onset of disease (years), mean±SD	25.9±9.3	22.7±9.3	0.152
Duration of psoriasis (years), mean±SD	9.1±7.4	12.9±7.4	**0.037**
Previous treatment for psoriasis, n (%)	32 (100)	40 (100)	–
Cumulative dose of Lindioil (g), mean±SD	845.3±690.7	766.8±692.1	0.633
PASI score at baseline, mean±SD	11.9±4.8	11.7±3.8	0.854
BSA% at baseline, mean±SD	9.6±5.6	11.3±6.3	0.232
Treatment response at week 8			
PASI improvement, mean±SD	46.3±33.9	64.8±31.0	**0.018**
PASI 75, n (%)	10 (31.3)	24 (60.0)	**0.019**
BSA improvement, mean±SD	28.6±63.5	55.2±41.5	**0.036**
Treatment response at week 12			
PASI improvement, mean±SD	50.0±34.2	67.8±28.3	**0.018**
PASI 75, n (%)	10 (31.3)	20 (50.0)	0.150
BSA improvement, mean±SD	33.6±64.1	62.0±41.2	**0.026**
Treatment response at week 20			
PASI improvement, mean±SD	51.6±35.3	70.4±29.8	**0.017**
PASI 75, n (%)	10 (31.3)	21 (52.5)	0.095
BSA improvement, mean±SD	38.1±65.6	65.1±44.1	**0.041**
Adverse event	3 (9.4)	2 (5.0)	0.650

Notes: **P* value was calculated by Fisher's exact test or independent two-tailed *t*-test. Bold text was presented to emphasize *P* value<0.05.

(mean 12.9 years vs 9.1 years, *P*=0.037). No other significant differences in clinical baseline parameters were identified between *HLA-Cw*01:02* groups. The percentage of *HLA-Cw*01:02*-negative patients who achieved PASI 75 (good responders) was higher than that of *HLA-Cw*01:02*-positive patients (60.0% vs 31.3%, *P*=0.019) at week 8, and *HLA-Cw*01:02*-negative patients had better PASI improvements (score improvement 64.8% vs 46.3%, *P*=0.018) and BSA improvements (55.2% vs 28.6%, *P*=0.036) after 8 weeks of Lindioil treatment. *HLA-Cw*01:02*-negative patients also showed significantly larger improvements in mean PASI and BSA after 12 (PASI: 67.8% vs 50.0%, *P*=0.018; BSA: 62.0% vs 33.6%, *P*=0.026) and 20 weeks of treatment (PASI: 70.4% vs 51.6%, *P*=0.017; BSA: 65.1% vs 38.1%, *P*=0.041).

We then analyzed the *HLA-Cw*06:02* allele (Table 4 and Figure 1D–F) and found that 13 patients (18.1%) were *HLA-Cw*06:02*-positive, and 59 patients (81.9%) were *HLA-Cw*06:02*-negative. No significant differences were identified in any clinical baseline parameters between these two groups. The percentage of *HLA-Cw*06:02*-positive patients who achieved PASI 75 (good responders) was higher than the percentage of *HLA-Cw*06:02*-negative patients (76.9% vs 40.7%, *P*=0.029), and *HLA-Cw*06:02*-positive patients presented with larger improvements in PASI (74.6% vs 52.6%, *P*=0.005) and BSA (73.3% vs 36.8%, *P*<0.001) after 8 weeks of Lindioil treatment. In addition, we observed that *HLA-Cw*06:02*-positive patients showed significantly larger improvements in mean PASI and BSA after 12 (PASI: 75.0% vs 56.5%, *P*=0.013; BSA: 79.3% vs 42.7%, *P*=0.001) and 20 weeks of treatment (PASI: 81.4% vs 57.8%, *P*=0.001; BSA: 84.9% vs 46.1%, *P*<0.001). Similarly, *HLA-Cw*06:02*-positive patients achieved PASI 75 more frequently at all time points, although no significant difference was observed between the two groups at week 20. We also analyzed the relationships between response outcomes, AEs, and HLA-Cw alleles and found no significant associations (Supplementary Table S4).

The cytokine analysis showed that patients who achieved PASI 75 after 8 weeks of treatment presented with lower baseline plasma IL-17 expression levels than those who did not achieve PASI 75 after 8 weeks (11.28 pg/mL vs 15.82 pg/

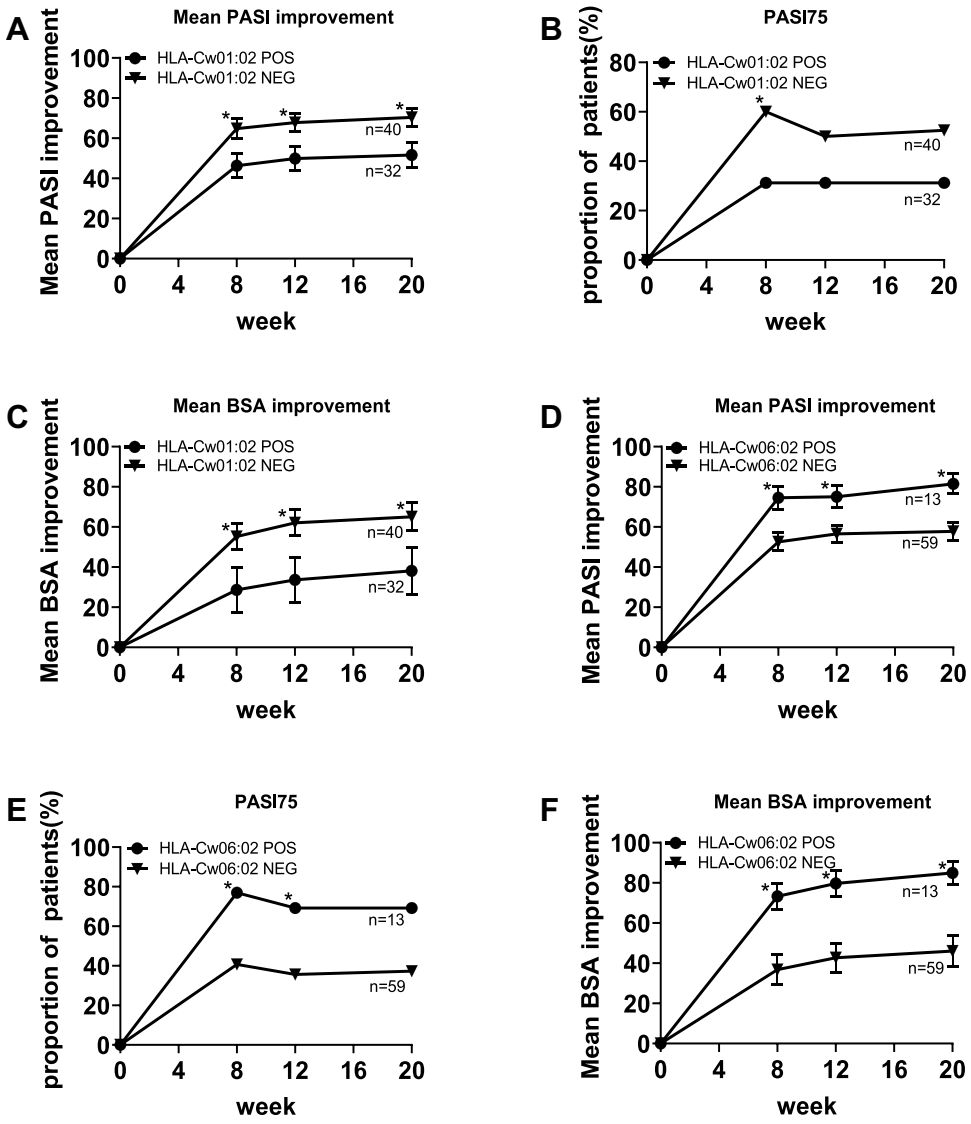

Figure 1 Clinical response to Lindioil stratified by the presence of HLA-Cw 01:02 and HLA-Cw 06:02. (**A**) shows that HLA-Cw 01:02 negative patients had significantly better mean PASI improvement than HLA-Cw 01:02 positive at week 8, 12, and 20. (**B**) shows that significantly more proportion of HLA-Cw 01:02 negative patients achieved PASI75 than HLA-Cw 01:02 positive at week 8. (**C**) shows that HLA-Cw 01:02 negative patients had significantly better mean BSA improvement than HLA-Cw 01:02 positive at week 8, 12, and 20. (**D**) shows that HLA-Cw 06:02 positive patients had significantly better mean PASI improvement than HLA-Cw 06:02 negative at week 8, 12, and 20. (**E**) shows that significantly more proportion of HLA-Cw 06:02 positive patients achieved PASI75 than HLA-Cw 06:02 negative at week 8 and 12. (**F**) shows that HLA-Cw 06:02 positive patients had significantly better mean BSA improvement than HLA-Cw 06:02 negative at week 8, 12, and 20. Error bars show standard error. *P < 0.05.

Abbreviations: PASI, Psoriasis Area and Severity Index; BSA, body surface area; POS, positive; NEG, negative.

mL, *P*=0.05, Figure 2), whereas no significant differences were identified in other baseline cytokine expression levels, including IL-6, IL-22, IL-23 and TNF-α (Supplementary Table S5). No significant differences in baseline cytokine levels were observed between patients who did and did not achieve PASI 50 after 8 weeks of treatment.

Discussion

The application of predictive genetic markers to treatment selection could reveal whether a given treatment is likely to result in a good response for the patient. Psoriasis is a chronic, long-term disease with a long average duration, and the identification of more effective treatments is a high priority for patients. *HLA-Cw*06:02* has previously been identified as a major genetic susceptibility allele for psoriasis,[24] and the findings of the present study are consistent with previous genetic findings. Both methotrexate and ustekinumab have been shown to be more effective in *HLA-Cw*06:02*-positive patients,[7,8,25] whereas no

Table 4 Demographic and Clinical Characteristics of *HLA-Cw*06:02* Positive/Negative Patients Treated with Lindioil

Demographic Data	*Cw*06:02* Positive (n=13)	*Cw*06:02* Negative (n=59)	*P* value*
Demographic characteristics			
Male, n (%)	5 (38.5)	38 (64.4)	0.119
Mean Age (years), mean±SD	35.0±8.3	35.4±8.3	0.883
Body Mass Index (kg/m^2), mean±SD	22.8±4.6	25.0±4.0	0.119
Clinical characteristics			
Age at onset of disease (years), mean±SD	22.8±7.5	24.4±9.8	0.569
Duration of psoriasis (years), mean±SD	12.2±7.9	11.0±7.6	0.587
Previous treatment for psoriasis, n (%)	13 (100)	59 (100)	–
Cumulative dose of Lindioil (g), mean±SD	792.9±579.8	803.6±713.7	0.96
PASI score at baseline, mean±SD	11.2±4.7	11.9±4.2	0.558
BSA% at baseline, mean±SD	10.8±7.8	10.5±5.6	0.846
Treatment response at week 8			
PASI improvement, mean±SD	74.6±20.6	52.6±34.4	**0.005**
PASI 75, n (%)	10 (76.9)	24 (40.7)	**0.029**
BSA improvement, mean±SD	73.3±23.0	36.8±56.3	**<0.001**
Treatment response at week 12			
PASI improvement, mean±SD	75.0±19.8	56.5±33.5	**0.013**
PASI 75, n (%)	9 (69.2)	21 (35.6)	**0.033**
BSA improvement, mean±SD	79.7±24.1	42.7±56.7	**0.001**
Treatment response at week 20			
PASI improvement, mean±SD	81.4±18.1	57.8±34.7	**0.001**
PASI 75, n (%)	9 (69.2)	22 (37.3)	0.061
BSA improvement, mean±SD	84.9±21.5	46.1±58.8	**<0.001**
Adverse event	1 (7.7)	4 (6.8)	1

Notes: **P* value was calculated by Fisher's exact test or independent two-tailed *t*-test. Bold text was presented to emphasize *P* value<0.05.

relationships have been identified between *HLA-Cw6* status and treatment response to secukinumab,[26] etanercept, or infliximab.[27] Another biologic agent, adalimumab, was found to result in a poorer response than ustekinumab in *HLA-Cw*06:02*-positive patients.[28] Moreover, fewer methotrexate treatment-related AEs were reported among *HLA-Cw*06:02*-positive patients.[7] *HLA-Cw*01* frequently appears among patients with psoriasis in several countries, particularly Asian countries, with a frequency ranging from 2.4% to 27%.[29–33] Cai and others highlighted that *HLA-Cw*01:02* is a psoriasis-associated allele in Southern Han Chinese. Moreover, *HLA-Cw*01:02*-positive patients tended to show no response to

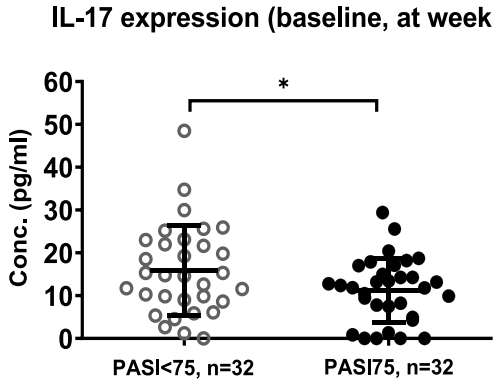

Figure 2 The plasma IL-17 expression before Lindioil treatment among patient groups achieving different PASI at week 8. It shows that patients achieving PASI>75 presented lower IL-17 expression than those with PASI<75. Error bars show standard deviation. *P < 0.05.
Abbreviations: IL-17, interleukin 17; PASI, Psoriasis Area and Severity Index.

conventional therapies.[30,34] In our study, 32 of 72 (44.4%) participants were *HLA-Cw*01:02*-positive, and all patients had received conventional psoriasis treatment prior to our study. The relatively high *HLA-Cw*01:02* frequency of our study population may indicate a tendency for this population to experience an overall poor response to conventional therapies, highlighting the need to develop alternative treatment strategies. The identification of gene status among patients with psoriasis can improve personalized medicine approaches, preventing the waste of time and money on treatments that are unlikely to be effective. However, currently, no evidence exists to support a relationship between genetic factors and the treatment response to Lindioil, a fast-acting topical therapy for psoriasis patients. In this study, we used the DNA database from our previous clinical trial[11] to analyze the correlation between HLA-Cw allele status and the treatment response to Lindioil.

We identified that *HLA-Cw*06:02*-positive and *HLA-Cw*01:02*-negative patients were more likely to be good responders to Lindioil treatment than patients with other allele presentations. Our results further revealed that *HLA-Cw*06:02*-positive and *HLA-Cw*01:02*-negative patients showed better responses to Lindioil therapy than patients with other allele presentations across several response evaluations, including mean PASI improvement, the proportion of patients achieving PASI 75, and mean BSA improvement. When examining clinical baseline parameters, *HLA-Cw*01 :02*-negative patients were found to have longer psoriasis history; however, no association was reported between disease duration and treatment response by Edson-Heredia and others.[35] Among those with good responses to Lindioil treatment, 73.5% were *HLA-Cw*01:02*-negative or *HLA-Cw*06:02*-positive, suggesting that both the *HLA-Cw*01:02* and *HLA-Cw*06:02* alleles participate in the pharmacological mechanism underlying the effects of Lindioil. The finding that *HLA-Cw*01:02*-positive patients showed a worse response to Lindioil than *HLA-Cw*01:02*-negative patients is consistent with one alefacept response study.[30] Further study remains necessary to determine the pathogenic mechanisms associated with HLA-Cw*01:02 in psoriasis and the biogenetic mechanism that determines the response of patients with *HLA-Cw*01:02* or *HLA-Cw*06:02* alleles to Lindioil treatment.

The reduced incidence of treatment-related AEs is among the advantages of topical Lindioil use. Among the 6 patients who experienced Lindioil-related AEs, 2 were *HLA-Cw*01:02*-negative, and only 1 was *HLA-Cw*06:02*-positive. None of these 6 patients were both *HLA-Cw*01:02*-negative and *HLA-Cw*06:02*-positive. Although an insufficient number of cases experienced AEs for statistical analyses, our findings suggested that *HLA-Cw*06:02*-positive and *HLA-Cw*01:02*-negative patients were less likely to suffer from treatment-related AEs than patients with other allelic combinations. We also identified that the IL-17 signaling pathway is involved in the treatment response to Lindioil. Significantly decreased plasma levels of IL-6, IL-17, IL-22, IL-23, and TNF-α were observed in good responders compared with poor responders. The primary active compounds in indigo naturalis have been reported to attenuate IL-17A expression, and our results indicate that topical treatment with Lindioil ointment in patients with psoriasis may also decrease the plasma expression levels of psoriasis-related cytokine expression. These findings indicate that psoriasis can be regarded as a systemic disease, and Lindioil may significantly decrease the Th17 cell population or associated pathways. In addition, patients who achieved PASI 75 after 8 weeks of Lindioil treatment presented with lower baseline plasma IL-17 expression; however, IL-17 was the only psoriasis-related cytokine found to be significantly reduced in patients with better treatment response, making this unlikely to reflect an overall reduction in inflammation status among the good-responder population. However, patients with a poor response to Lindioil may require combination treatments using other Medicines (eg, traditional immunosuppressants or anti-IL-23/IL-12 biologic agents) or therapeutic approaches (phototherapy) to improve treatment response and suppress psoriasis-related cytokine expression.

One of the limitations of this study was the number of cases recruited at two hospital centers. Not all patients with psoriasis who were treated during the trial study period agreed to the extraction of sufficient blood samples for the performance of genetic analyses; however, all patients who received a genetic analysis were included in our cohort study. We were unable to demonstrate any significant correlation between HLA-Cw allele status and cytokine expression patterns among patients with psoriasis (data not shown), and only five types of psoriasis-related cytokines were investigated. Cytokine expression analysis performed using skin specimens obtained from psoriasis lesion might be more strongly correlated with disease severity and treatment response than the cytokine expression pattern in plasma. Large-scale studies remain necessary to fully evaluate the influence of *HLA-Cw* allele status and cytokine expression patterns on the response to Lindioil treatment.

Conclusion

In conclusion, *HLA-Cw*01:02*-negative and *HLA-Cw*06:02*-positive status and lower plasma IL-17 levels were correlated with a good Lindioil treatment response among psoriasis patients. Our study provides predictive markers for treatment response which may lead to improvements in the topical Lindioil treatment in patients with psoriasis.

Data Sharing Statement

The datasets generated during and/or analysed during the current study are available from first author, Yin-Ku Lin, on reasonable request.

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Author Contributions

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare no conflicts of interest for this work and that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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