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ERAP1 and *ERAP2* gene variants as potential clinical biomarkers of anti-interleukin-17A response in psoriasis vulgaris

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Abstract

Background Interleukin (IL)-17A is a proinflammatory cytokine that plays an essential role in the development of psoriasis. Although treatment with anti-IL-17A monoclonal antibodies has demonstrated high efficacy in patients with psoriasis, not all patients respond equally well, highlighting the need for biomarkers to predict treatment response. Specific single-nucleotide polymorphisms (SNPs) in the genes encoding endoplasmic reticulum aminopeptidases 1 and 2 (ERAP1 and ERAP2) have been associated with psoriasis and other immune-mediated diseases.

Objectives To investigate the association between the *ERAP1* and *ERAP2* genotypes and response to secukinumab treatment in patients with psoriasis.

Methods In total, 75 patients with plaque psoriasis were included. All patients were genotyped for the *ERAP1* rs27524, rs27044, rs30187, rs2287987 and rs26653 SNPs, the *ERAP2* rs2248374 SNP, and the status of the human leucocyte antigen *HLA-C*06:02* gene.

Results Our results demonstrated that individuals with specific *ERAP1* and *ERAP2* genotypes had a considerably lower response rate to secukinumab treatment. Patients with the *ERAP2* rs2248374 GG genotype had a more than sixfold increased risk of treatment failure compared with patients with the rs2248374 AG or AA genotypes. Stratifying for *HLA-C*06:02* status, the *ERAP2* GG genotype pointed towards an increased risk of treatment failure among *HLA-C*06:02*-positive patients, although this was not statistically significant.

Conclusions Taken together, this unique study breaks new ground by identifying distinct *ERAP1* and *ERAP2* gene variants that may serve as potential biomarkers for predicting the treatment response to secukinumab in patients with psoriasis. Notably, our data extend existing knowledge by linking specific *ERAP1* and *ERAP2* gene variants to treatment outcome.

What is already known about this topic?

- Interleukin (IL)-17A is a proinflammatory cytokine implicated in the development of psoriasis.
- Anti-IL-17A monoclonal antibodies have demonstrated high efficacy in treating psoriasis, but responses vary among patients.
- Endoplasmic reticulum aminopeptidases 1 and 2 (ERAP1 and ERAP2) have been associated with psoriasis and other immune-mediated diseases. They are involved in processing endogenous peptides for human leucocyte antigen class I-mediated presentation to $CD8^+$ T cells.

What does this study add?

- This study investigates the association between *ERAP1* and *ERAP2* genotypes and response to secukinumab treatment in patients with psoriasis.
- The study found that specific *ERAP1* and *ERAP2* genotypes are associated with a lower response rate to secukinumab treatment.
- *HLA-C*06:02* status may influence the effect of the *ERAP2* genotype on anti-IL-17A response.
- *ERAP1* and *ERAP2* gene variants may serve as potential clinical biomarkers for predicting secukinumab treatment response in patients with psoriasis.

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Introduction

Psoriasis is a chronic, inflammatory, immune-mediated skin disease globally affecting 2–4% of the human population.[1] The pathogenesis is complex and not fully understood.[2,3] It involves various immune cells, cytokines and genetic factors.[4] Interleukin (IL)-17A is a proinflammatory cytokine produced by several immune cells, including T helper 17 cells, γδ T cells, and innate lymphoid cells.[5,6]

IL-17A binds to its receptor, IL-17RA, located on keratinocytes and other skin cells, leading to the activation of downstream signalling pathways that promote inflammation. Several studies have demonstrated IL-17A to be a critical contributor to the development of psoriasis.[7–10] This is further supported by clinical evidence showing that treatment with antibodies targeting IL-17A results in a high degree of efficacy in the management of psoriasis.[11]

Anti-IL-17A monoclonal antibody treatments approved for psoriasis vulgaris include secukinumab and ixekizumab. Although these treatments are highly efficacious, not all patients with psoriasis respond equally well to these therapies, with some patients demonstrating inadequate response or loss of response over time.[12,13] Therefore, there is a growing need for biomarkers to predict the treatment response in order to optimize and stratify therapy for people with psoriasis.

Psoriasis is a multifactorial disease, where a substantial part of an individual's risk relies on genetic factors. More than 80 risk loci have been identified across the genome, with the most predominant genetic risk factor being the human leucocyte antigen *HLA-C*06:02* allele, which is present in up to 60% of people with psoriasis.[14] As a part of the major histocompatibility complex class I (MHC-I), the primary function of HLA-C is presentation of endogenous peptides to responding $CD8^+$ T cells.[15] This results in activation of $CD8^+$ T cells and subsequent inflammation.

Other genetic risk factors are allelic variants in the genes coding for endoplasmic reticulum aminopeptidases 1 and 2 (ERAP1 and ERAP2).[16] ERAP1 and ERAP2 are members of the aminopeptidase family and are involved in the processing of endogenous peptides for HLA class I-mediated presentation to the immune system.[17] Besides psoriasis, *ERAP1* and *ERAP2* polymorphisms are associated with other immune-mediated diseases with specific HLA backgrounds, such as ankylosing spondylitis.[16,18]

The ERAP2 protein has two isoforms due to a specific single-nucleotide polymorphism (SNP) in *ERAP2*, rs2248374 (alleles A and G). The G allele is known to cause nonsense-mediated decay of mRNA because of alternative splicing, thus resulting in no ERAP2 protein being produced.[19] One-quarter of individuals are GG homozygous and thus express no ERAP2 protein. One-half are AG heterozygous and express reduced amounts of the protein, and the remaining 25% are AA homozygous and express substantial amounts of the protein.[19]

The exact role of ERAP1 and ERAP2 in the pathogenesis of psoriasis is still not fully understood, nor is their relationship with treatment response and disease progression in patients receiving biologic therapies, such as anti-IL-17A treatment. Nonetheless, a study demonstrated that the *ERAP2* rs2248374 SNP and certain haplotypes in the *ERAP1* rs27524, rs27044, rs30187, rs2287987 and rs26653 SNPs were associated with increased susceptibility to psoriasis, and that this susceptibility was further enhanced when the patients also carried the *HLA-C*06:02* allele.[16]

In this study, we aimed to investigate the association between the *ERAP1* rs27524, rs27044, rs30187, rs2287987 and rs26653, and the *ERAP2* rs2248374 genotypes and response to secukinumab therapy. We hypothesized that specific *ERAP1* and *ERAP2* variants may predict secukinumab treatment outcome in patients with psoriasis.

Patients and methods

Study patients

Patients with psoriasis ($n=51$) who had received or were receiving treatment with secukinumab were enrolled. The study was conducted at the Department of Dermatology at Aarhus University Hospital between May 2021 and January 2022, and patients were enrolled during their routine visits in the clinic. Patients ≥ 18 years of age with a diagnosis of moderate-to-severe psoriasis, defined as being eligible for treatment with biologics based on the criteria in Denmark, were evaluated for eligibility. All patients voluntarily donated 2.5 mL of whole blood, which was collected by a laboratory technician using PAXgene Blood DNA tubes for *in vitro* diagnostic purposes (Qiagen, Hilden, Germany). The tubes were inverted eight times and stored at −20 °C overnight before being permanently stored at −150 °C until further use. In total, 51 blood samples were collected.

To expand our cohort, we included an additional 24 patients who had previously donated blood in 2019 at the Department of Dermatology, Aarhus University Hospital. They were receiving or had received treatment with secukinumab and had been genotyped for *ERAP2* rs2248374. For 5 of these 24 additional patients, *HLA-C*06:02* genotyping was not possible as the collected blood had been used for other purposes. None of these patients were genotyped according to the chosen *ERAP1* SNPs.

All patients provided written informed consent for use of their whole blood for scientific research in accordance with the Declaration of Helsinki. The project was approved by the Central Denmark regional ethics committee (no. M-2016-151-16).

Data extraction of study patients

Patient characteristics were obtained by reviewing the clinical records of the included patients using the electronic patient records at the hospital. Characteristics were collected at the date from the record that corresponded with the date of blood sampling. The following predefined clinical criteria were used to classify patients as either responders or nonresponders. Patients who had experienced sufficient treatment with secukinumab for ≥ 24 months were considered responders to treatment. Patients who experienced no or insufficient treatment response or experienced a loss of efficacy before 24 months after initiating treatment with secukinumab were considered nonresponders to treatment. Nonresponders were further investigated to assess whether

failure of treatment was due to no treatment response or loss of effect, or if it was due to adverse events, infection or other diseases.

DNA extraction and purification from whole blood

The QIAamp® DSP DNA Blood Mini Kit (Qiagen) was used to extract genomic DNA from 200 μL whole blood using the spin procedure according to the standard protocol. DNA samples were stored at −20 °C for later use.

Single-nucleotide polymorphism genotyping

Genotyping of SNPs in the *ERAP1* (rs27524, rs27044, rs30187, rs2287987 and rs26653), *ERAP2* (rs2248374) and *HLA-C* (rs4406273) genes was conducted using TaqMan® Predesigned SNP Genotyping Assays (Thermo Fisher Scientific Inc., Waltham, MA, USA). The assay IDs are available in Table S1 (see Supporting Information). *HLA-C*06:02* rs4406273 has previously been validated as a single SNP surrogate for genotyping *HLA-C*06:02* and was therefore selected.[20] Real-time genotyping polymerase chain reaction (PCR) was carried out on the StepOnePlus™ Real-Time PCR System (Thermo Fisher Scientific Inc.). Allelic discrimination plots were obtained through data analysis using the StepOnePlus™ software (Thermo Fisher Scientific Inc.).

Statistical analysis

Baseline characteristics were tested for significant differences between groups using the χ^2-test and Fisher's exact test for categorial variables. Hardy–Weinberg equilibrium analysis was carried out using PLINK software (version 1.90 beta 7.1).[21] To compare continuous data, the *t*-test was used for normally distributed data; otherwise, a Mann–Whitney rank sum test was used. Statistical analysis was conducted using R software (http://www.r-project.org; version 4.2.1). To determine the effects of baseline characteristics and *ERAP1* and *ERAP2* genotypes on a positive response, the R statistics logistic regression generalized model was used to conduct both univariate and multivariable analyses. Linkage disequilibrium (LD) analysis and haplotype association analysis were conducted using the SHEsis software platform (http://analysis.bio-x.cn).[22,23] Haplotype frequencies between groups were analysed using the χ^2-test. Odds ratios (ORs) and corresponding 95% confidence intervals (CIs) corresponding to haplotypes were calculated as a measure of effect size. *P*-values < 0.05 were considered statistically significant.

Results

Characteristics of study patients

All tested polymorphisms in the *ERAP1*, *ERAP2* and *HLA-C* genes were in Hardy–Weinberg equilibrium (Table S1; see Supporting Information). The main clinical characteristics of the patients with psoriasis treated with secukinumab are summarized in Table 1. In total, 75 participants were enrolled, including 53 men and 22 women, with a mean age of 47 years. Based on predefined clinical criteria, 43 patients (57%) were classified as responders to secukinumab treatment, while 32 (43%) were nonresponders. Two clinical characteristics displayed a statistically significant difference between responders and nonresponders. Firstly, nonresponders were more likely to currently smoke ($P=0.02$; Table 1), which is consistent with findings in another Danish study from 2021.[24] Also, nonresponders had been treated with a higher number of different biologic treatments before receiving secukinumab treatment ($P<0.001$; Table 1).

Distribution of *ERAP1* and *ERAP2* polymorphisms according to treatment response

When investigating the association of *ERAP2* gene variants with response to secukinumab, the results demonstrated that the risk of treatment failure was significantly lower for the rs2248374 AG genotype than for the reference genotype rs2248374 GG (OR 0.32, 95% CI 0.10–0.99, $P=0.047$;

Table 1 Main characteristics of secukinumab-treated patients with psoriasis and differences between responders and nonresponders

	All patients (*n* = 75)	Nonresponders (*n* = 43, 57%)	Responders[a] (*n* = 32, 43%)	*P*-value
Sex, male; *n* (%)	53 (71)	27 (63)	26 (81)	0.08
Age, years; mean (SD)	47 (14)	48 (13)	46 (14)	0.58
Age of onset, years; mean (SD)	23 (12)	23 (13)	23 (11)	> 0.99
Body mass index, kg m^{-2}; mean (SD)	32 (7)	33 (6)	30 (8)	0.18
Number of biologic treatments before secukinumab, mean (SD)	1.43 (1.9)	2.02 (2.1)	0.63 (1.2)	**< 0.001**
Smoking status, *n* (%)[b]				
Never	36 (49)	19 (44)	17 (55)	
Previous	19 (26)	8 (19)	11 (35)	
Current	19 (26)	16 (37)	3 (10)	**0.02**
Presence of psoriatic arthritis, *n* (%)	23 (31)	12 (28)	11 (34)	0.55
*HLA-C*06:02*-positive, *n* (%)	38 (51)	23 (53)	15 (46)	0.57
ERAP2 genotype, *n* (%)				
GG	25 (33)	18 (42)	7 (22)	
AG	31 (41)	14 (33)	17 (53)	
AA	19 (25)	11 (26)	8 (25)	0.13

[a]Responders were patients who sustained treatment for > 24 months. [b]Data missing for one responder. *P*-values < 0.05 are significant and annotated in bold.

Table 2 Distribution of *ERAP2* and *ERAP1* single-nucleotide polymorphisms according to secukinumab treatment response

Genotype/allele	Nonresponders ($n=34$, 67%)	Responders[a] ($n=17$, 33%)	*P*-value	OR (95% CI)
ERAP2 rs2248374				
GG	18 (42)	7 (22)	–	1.00[b]
AG	14 (33)	17 (53)	**0.047**	0.32 (0.10–0.99)
AA	11 (26)	8 (25)	0.38	0.54 (0.15–1.89)
G (risk allele)	50 (58)	31 (48)	0.24	1.48 (0.77–2.84)
ERAP1 rs27524				
GG	14 (41)	4 (24)	–	1.00[b]
AG	15 (44)	5 (29)	0.84	0.86 (0.19–3.85)
AA	5 (15)	8 (47)	**0.032**	0.18 (0.04–0.86)
G (risk allele)	43 (63)	13 (38)	**0.018**	2.78 (1.19–6.50)
ERAP1 rs27044				
CC	17 (50)	5 (29)	–	1.00[b]
CG	14 (41)	9 (53)	0.24	0.46 (0.12–1.68)
GG	3 (9)	3 (18)	0.20	0.29 (0.04–1.94)
C (risk allele)	48 (71)	19 (56)	0.14	1.89 (0.81–4.45)
ERAP1 rs30187				
CC	16 (47)	4 (24)	–	1.00[b]
CT	14 (41)	8 (47)	0.25	0.44 (0.11–1.77)
TT	4 (12)	5 (29)	0.065	0.20 (0.04–1.11)
C (risk allele)	46 (68)	16 (47)	**0.047**	2.35 (1.01–5.47)
ERAP1 rs2287987	($n=33$, 67%)	($n=16$, 33%)		
CC	11 (33)	2 (13)	–	1.00[b]
CT	11 (33)	6 (38)	0.23	0.33 (0.05–2.03)
TT	11 (33)	8 (50)	0.12	0.25 (0.04–1.45)
C (risk allele)	33 (50)	10 (31)	0.082	2.20 (0.90–5.36)
ERAP1 rs26653	($n=33$, 66%)	($n=17$, 34%)		
CC	15 (45)	4 (24)	–	1.00[b]
CG	15 (45)	11 (65)	0.14	0.36 (0.09–1.40)
GG	3 (9)	2 (12)	0.46	2.00 (0.32–12.6)
C (risk allele)	45 (68)	19 (56)	0.23	1.69 (0.72–3.79)

The data are presented as *n* (%). CI, confidence interval; OR, odds ratio. [a]Responders were patients who sustained treatment for > 24 months. [b]Reference group. *P*-values < 0.05 are significant and annotated in bold.

Table 2). No significant difference was observed between the rs2248374 GG and AA genotypes. Additionally, there were no observed differences in minor allele frequencies between responders and nonresponders.

Likewise, in the context of *ERAP1* gene variants, the risk of treatment failure was significantly reduced for individuals carrying the rs27524 AA genotype compared with the reference genotype rs27524 GG (OR 0.18, 95% CI 0.04–0.86, $P=0.032$; Table 2). Minor allele frequencies revealed a significant difference between responders and nonresponders in *ERAP1* rs27524 ($P=0.018$) and rs30187 ($P=0.047$; Table 2). For the remaining three *ERAP1* SNPs (rs27044, rs2287987 and rs26653) no significant differences in allele frequencies were observed between the two groups.

Association of the *ERAP1* and *ERAP2* genotypes with treatment response

To address potential predictors of secukinumab treatment failure, both univariable and multiple logistic regression analyses were conducted, the latter to adjust for potential covariates. No differences in risk of treatment failure were observed between *ERAP2* rs2248374 genotypes. In the univariable analysis, the only significant difference in risk of treatment failure was observed in the *ERAP1* rs27524 SNP, when comparing the combined GG and AG genotype to the AA genotype ($P=0.02$; Table 3). However, when adjusting for potential covariates, patients with the *ERAP2* rs2248374 GG genotype were found to have a more than sixfold increased risk of treatment failure compared with patients with the rs2248374 AG or AA genotype (OR 6.26, 95% CI 1.17–33.5, $P=0.03$; Table 3).

Excluding patients who discontinued treatment due to infection or adverse events resulted in an almost ninefold increased risk of treatment failure (OR 8.84, 95% CI = 1.06–73.8, $P=0.04$; Table 3). Furthermore, combining the rs2248374 GG and AA genotypes in the same group resulted in a more than ninefold increased risk of treatment failure compared with the rs2248374 AG genotype (OR 9.54, 95% CI 1.19–76.6, $P=0.03$; Table 3). This suggests that the rs2248374 AG genotype may be protective against secukinumab treatment failure. Also, genotypes in the *ERAP1* SNPs revealed significant differences in the multivariable analysis, displaying significant results for all SNPs except the *ERAP1* rs26653 SNP (Table 3).

In order to investigate whether the observed association between the *ERAP2* SNP and treatment response was specific to secukinumab treatment or if it extended to other drugs with different mechanisms of action, we conducted the same analyses on two already existing cohorts of patients with psoriasis receiving adalimumab ($n=61$) and ustekinumab ($n=59$). These cohorts were previously genotyped for *ERAP2*, but as the collected material was no longer available, genotyping for *ERAP1* SNPs was unfortunately not possible. The main characteristics of the patients, and the distributions of *ERAP2* SNPs among those treated

Table 3 *ERAP2* and *ERAP1* genotypes and risk of secukinumab treatment failure

	Univariable analysis[a]		Multivariable analysis[b]	
	***P*-value**	**OR (95% CI)**	***P*-value**	**OR (95% CI)**
ERAP2 rs2248374				
All patients ($n=75$)				
GG/(AG+AA)	0.07	2.57 (0.90–7.36)	**0.03**	6.26 (1.17–33.5)
(GG+AG)/AA	0.95	0.97 (0.33–2.83)	0.94	1.06 (0.23–4.90)
(GG+AA)/AG	0.08	2.35 (0.90–6.12)	0.05	4.11 (0.95–17.8)
Patients excluding those with failure due to infection or adverse events ($n=61$)				
GG/(AG+AA)	0.17	2.18 (0.69–6.88)	**0.04**	8.84 (1.06–73.8)
(GG+AG)/AA	0.82	0.88 (0.27–2.81)	0.54	0.57 (0.09–3.54)
(GG+AA)/AG	0.15	2.15 (0.75–6.19)	**0.03**	9.54 (1.19–76.6)
ERAP1 rs27524				
All patients ($n=51$)				
GG/(AG+AA)	0.22	2.28 (0.59–8.74)	0.08	6.38 (0.77–52.7)
(GG+AG)/AA	**0.02**	5.16 (1.30–20.5)	**0.02**	402 (> 2.71)[c]
(GG+AA)/AG	0.31	0.53 (0.15–1.89)	0.07	0.18 (0.01–1.33)
Patients excluding those with failure due to infection or adverse events ($n=41$)				
GG/(AG+AA)	0.71	2.32 (0.56–9.68)	**0.02**	158 (> 1.56)[c]
(GG+AG)/AA	**0.04**	4.44 (1.01–19.5)	–	–
(GG+AA)/AG	0.42	0.58 (0.15–2.28)	0.37	0.29 (0.20–5.01)
ERAP1 rs27044				
All patients ($n=51$)				
CC/(CG+GG)	0.17	2.40 (0.67–8.57)	**0.02**	17.0 (1.32–218)
(CC+CG)/GG	0.37	2.21 (0.38–12.9)	0.20	5.82 (0.36–95.0)
(CC+GG)/CG	0.43	1.61 (0.48–5.35)	0.12	4.24 (0.63–28.4)
Patients excluding those with failure due to infection or adverse events ($n=41$)				
CC/(CG+GG)	0.12	2.84 (0.73–11.0)	–	–
(CC+CG)/GG	0.38	2.36 (0.33–16.9)	0.19	16.7 (> 0.21)[c]
(CC+GG)/CG	0.33	1.88 (0.51–6.89)	0.06	157 (> 0.65)[c]
ERAP1 rs30187				
All patients ($n=51$)				
CC/(CT+TT)	0.11	2.89 (0.76–11.0)	**0.04**	9.96 (1.09–90.9)
(CC+CT)/TT	0.13	3.13 (0.69–14.2)	**0.02**	94 (> 1.66)[c]
(CC+TT)/CT	0.69	1.27 (0.38–4.22)	0.73	1.39 (0.21–9.41)
Patients excluding those with failure due to infection or adverse events ($n=41$)				
CC/(CT+TT)	0.09	3.25 (0.79–13.5)	**0.02**	138 (> 1.67)[c]
(CC+CT)/TT	0.19	2.92 (0.56–15.2)	0.05	6634 (> 0.60)[c]
(CC+TT)/CT	0.54	1.48 (0.40–5.44)	0.22	5.09 (0.33–79.2)
ERAP1 rs2287987				
All patients ($n=49$)				
CC/(TC+TT)	0.14	3.50 (0.64–19.0)	**0.03**	13.8 (1.19–159)
(CC+CT)/TT	0.27	2.00 (0.57–6.98)	**0.04**	17.1 (1.02–287)
(CC+TT)/CT	0.29	1.20 (0.34–4.30)	0.87	1.20 (0.13–10.8)
Patients excluding those with failure due to infection or adverse events ($n=39$)				
CC/(TC+TT)	0.08	4.50 (0.78–26.1)	–	–
(CC+CT)/TT	0.13	2.83 (0.70–11.5)	–	–
(CC+TT)/CT	0.86	1.13 (0.29–4.44)	0.71	3.44 (0.10–125)
ERAP1 rs26653				
All patients ($n=50$)				
CC/(CG+GG)	0.14	2.71 (0.71–10.4)	0.49	1.83 (0.19–22.7)
(CC+CG)/GG	0.39	2.14 (0.37–12.5)	0.37	3.56 (0.20–62.0)
(CC+GG)/CG	0.37	1.71 (0.51–5.78)	0.71	0.65 (0.06–6.94)
Patients excluding those with failure due to infection or adverse events ($n=40$)				
CC/(CG+GG)	0.07	3.55 (0.85–14.9)	0.15	14.1 (0.33–608)
(CC+CG)/GG	0.83	2.25 (0.31–16.2)	0.06	1090 (> 0.58)[c]
(CC+GG)/CG	0.22	2.22 (0.59–8.32)	0.55	0.24 (0.00–33.2)

CI, confidence interval; OR, odds ratio. [a]OR (95% CI), unadjusted. [b]OR (95% CI), adjusted for age at time of blood sampling, age at onset, sex, body mass index, psoriatic arthritis status, smoking status, number of treatments before secukinumab and *HLA-C*06:02* status. [c]Lower limit of the 95% CI is given; upper limit is beyond the range of estimation. *P*-values < 0.05 are significant and annotated in bold.

Table 4 *ERAP2* genotype and risk of secukinumab treatment failure according to *HLA-C*06:02* status

	Patient ***HLA-C*06:02*** **status**			
	Negative (*n*=31)		**Positive (*n*=39)**	
ERAP2 **rs2248374 genotype**	***P*-value**	**OR (95% CI)[a]**	***P*-value**	**OR (95% CI)[a]**
GG/(AG+AA)	0.20	5.62 (0.34–92.7)	0.06	11.5 (0.77–173)
(GG+AG)/AA	0.20	1.39 (0.04–44.5)	0.85	0.81 (0.08–7.89)
(GG+AA)/AG	0.12	35.3 (0.30–4197)	0.09	5.29 (0.73–38.5)

CI, confidence interval; OR, odds ratio. [a]OR (95% CI), adjusted for age at time of blood sampling, age at onset, sex, body mass index, psoriatic arthritis status, smoking status, number of treatments before secukinumab and *HLA-C*06:02* status. *P*-values < 0.05 are significant.

with adalimumab and ustekinumab, are described in Tables S2–S5 (see Supporting Information). None of the *ERAP2* rs2248374 genotypes were associated with increased risk of adalimumab or ustekinumab treatment failure (Tables S6 and S7; see Supporting Information).

When stratifying the samples from secukinumab-treated patients based on *HLA-C*06:02* status, no significant difference in risk of treatment failure was observed for any of the *ERAP2* genotypes (Table 4). However, the estimated OR for risk of treatment failure was higher among *HLA-C*06:02*-positive patients. Here, a more than 11-fold increased risk of treatment failure was observed for the *ERAP2* rs2248374 GG genotype (OR 11.5, 95% CI 0.77–173, *P*=0.06; Table 4).

When stratifying based on sex, no significant associations between genotypes and secukinumab treatment were observed in male participants in the multiple logistic regression analysis, when comparing rs2248374 GG to the rs2248374 AG and AA genotypes (OR 4.86, 95% CI 0.78–30.2, *P*=0.08; Table S8; see Supporting Information). Interestingly, this analysis could not be conducted on female participants, as there were no women with the rs2248374 GG genotype in the responder group.

Association of *ERAP1* and *ERAP2* haplotypes with secukinumab treatment response

To further investigate the association between *ERAP1* and *ERAP2* SNPs and risk of secukinumab treatment failure, we conducted a haplotype association analysis on the six SNPs. Sixteen haplotypes with frequencies > 3% in either nonresponders or responders were found (Table 5). These represented 100% and 88% of haplotypes in the respective groups. Eight of these 16 haplotypes were present in both groups. The haplotype distribution differed significantly between nonresponders and responders (global *P*-value = 0.008; Table 5).

Haplotypes H2 (rs27524A, rs27044C, rs30187 T, rs2287987 T, rs26653G, rs2248374A) and H16 (rs27524G, rs27044G, rs30187 T, rs2287987C, rs26653G, rs2248374G) were more frequent in nonresponders (*P*=0.04 and *P*=0.047, respectively; Table 5). In contrast, haplotype H10 (rs27524G, rs27044C, rs30187C, rs2287987C, rs26653C, rs2248374G) was significantly more frequent in responders (*P*=0.005; Table 5).

LD analysis showed little or no LD between most genotypes. However, rs27524 and rs30187 were found to be in high LD with each other, and this was most pronounced among responders (Figure S1; see Supporting Information).

Discussion

Previous studies have indicated that specific genetic variations in *ERAP1* and *ERAP2* may influence psoriasis susceptibility and disease severity.[16,25] However, the effect of these genetic variants on treatment response has not been

Table 5 Estimated haplotype frequencies in nonresponders (NR) and responders (R)

Haplotype ID	*ERAP1*					*ERAP2*				
	rs27524	**rs27044**	**rs30187**	**rs2287987**	**rs26653**	**rs2248374**	**NR %**	**R %**	***P*-value**	**OR (95% CI)**
H1	A	C	C	T	C	A	9.9	1.6	0.09	5.85 (0.61–55.8)
H2	A	C	T	T	G	A	12.5	1.6	**0.04**	7.9 (0.85–74.2)
H3	A	G	C	T	C	A	0	3.1	0.28	–
H4	A	G	T	T	C	A	0	3.2	0.27	–
H5	G	C	C	C	C	A	11.3	17.3	0.31	0.52 (0.15–1.86)
H6	G	C	C	C	G	A	3.4	3.2	0.96	0.94 (0.09–10.0)
H7	G	C	C	T	C	A	9.4	6.2	0.70	1.35 (0.28–6.47)
H8	A	G	T	T	C	G	9.6	7.2	0.81	1.20 (0.26–5.43)
H9	A	G	T	T	G	G	20.7	12.1	0.39	1.64 (0.52–5.15)
H10	G	C	C	C	C	G	0	18.8	**0.005**	–
H11	G	C	C	C	G	G	0	4.4	0.20	–
H12	G	C	C	T	C	G	0	6.4	0.12	–
H13	G	C	C	T	G	G	6.6	2.9	0.47	2.06 (0.28–15.5)
H14	A	G	T	C	C	A	6.6	0	0.05	–
H15	A	G	C	C	C	G	3.1	0	0.18	–
H16	G	G	T	C	G	G	6.8	0	**0.047**	–

Global *P*-value for all haplotypes = 0.008 (χ^2 = 31.5, df = 15). Haplotypes with frequency < 0.03 in both NR and R have been omitted. CI, confidence interval; OR, odds ratio. *P*-values < 0.05 are significant and annotated in bold.

examined and is therefore unknown. In this study, we investigated the association between specific *ERAP1* and *ERAP2* genotypes and response to secukinumab treatment in a cohort of patients with psoriasis. Our investigation yields compelling evidence supporting a role for *ERAP1* and *ERAP2* gene variants in anticipating the response to secukinumab therapy in patients with psoriasis.

Looking specifically at the *ERAP2* gene variants, our findings suggest that the association was specific to secukinumab and not to biologics in general, although the mechanism behind this is unknown. Interestingly, we demonstrated that the GG genotype of *ERAP2* rs2248374 was associated with a lower response rate to secukinumab treatment than the AG and AA genotypes. Likewise, the CC genotype of both *ERAP1* rs27044 and rs30187 was associated with failure of secukinumab treatment, whereas the AA genotype of *ERAP1* rs27524 and the TT genotype of *ERAP1* rs2287987 were found to be protective of treatment failure.

The underlying mechanistic explanation for these observations is not clear. However, it is interesting that previous studies have demonstrated the importance of ERAP1 and ERAP2 in the processing of endogenous peptides for HLA class I-mediated presentation to the immune system.[26,27] Thus, it is possible that lack of functional ERAP1 and ERAP2 modifies the immunopeptidome, resulting in an immune response that is less prone to be responsive to secukinumab treatment. Further research is needed to confirm this hypothesis and elucidate the underlying biological mechanisms.

The *HLA-C*06:02* allele is known to be a strong genetic risk factor for developing psoriasis.[2,28] Previous studies have emphasized the significance of *HLA-C*06:02* in the context of MHC presentation, suggesting that individuals carrying this allele may exhibit altered antigen presentation processes.[29] In line with this, it is interesting that there is a noteworthy prevalence of the *HLA-C*06:02* allele in patients with the GG genotype. This suggests an epistatic interaction between *ERAP2* and *HLA-C*06:02* in the development of psoriasis and response to secukinumab therapy. Although no significant results were obtained, the difference between the estimated ORs may imply that *HLA-C*06:02*-positive patients with the *ERAP2* rs2248374 GG genotype have an even higher risk of not responding to secukinumab treatment.

In our sex-based stratification, we did not identify a significantly elevated risk among male patients. However, due to the absence of women with the *ERAP2* rs2248374 GG genotype in the responder group, a multivariable statistical analysis for this subgroup was precluded. Nonetheless, our data suggest that the association between *ERAP2* genotype and risk of treatment failure is more pronounced among women.

This current study may have significant clinical implications for psoriasis treatment, especially considering the varying response rates among patients with psoriasis to anti-IL-17A therapies such as secukinumab. We propose that *ERAP1* and *ERAP2* gene variants may be potential clinical biomarkers for predicting the response to secukinumab therapy in these patients. This insight could pave the way for a more personalized approach to the treatment of psoriasis, where patients with specific *ERAP1* and *ERAP2* genotypes may benefit from alternative biologics with different mechanisms of action.

A limitation of our study is the small sample size, which may have limited our ability to detect significant differences in response rates between the different genotypes. A larger study with a more diverse patient population is needed to confirm our findings. Additionally, we did not measure serum levels of IL-17A, anti-IL-17A or other factors, which could have provided further insights into the relationship between *ERAP1* and *ERAP2* gene variants and response to secukinumab therapy. Baseline Psoriasis Area and Severity Index (PASI) score represents a significant clinical characteristic as a measure of disease severity at secukinumab treatment initiation. However, it was unavailable at the time of treatment initiation for most patients, which prohibits utilizing PASI for the analyses in the current study.

Taking the results together, this study demonstrates that the presence of specific SNPs in the *ERAP1* and *ERAP2* genes increases the risk of treatment failure in patients with psoriasis treated with secukinumab. Larger studies with more diverse patient populations should be conducted to confirm our findings and investigate the potential epistatic interactions between *ERAP1* and *ERAP2* and other psoriasis-associated genes, such as *HLA-C*06:02*. Such studies could lead to the development of a clinical biomarker panel including *ERAP1*, *ERAP2* and other genetic variants, as well as phenotypical, biochemical and molecular data, to predict treatment response in patients with psoriasis. This will ultimately lead to more personalized and effective treatment options for psoriasis.

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Conflicts of interest

TB has served as a paid speaker for Eli Lilly and LEO Pharma. LI has served as a consultant and/or paid speaker for and/or participated in clinical trials sponsored by AbbVie, Almirall, Amgen, AstraZeneca, BMS, Boehringer Ingelheim, Celgene, Centocor, Eli Lilly, Janssen Cilag, Kyowa, LEO Pharma, MSD, Novartis, Pfizer, Regranion, Samsung, UCB and Union Therapeutics. LI is also employed by MC2 Therapeutics A/S. CJ has served as a consultant and/or paid speaker for AbbVie, Eli Lilly, LEO Pharma and L'Oréal.

Data availability

The data underlying this article will be shared upon reasonable request to the corresponding author.

Ethics statement

All patients provided written informed consent for the use of their whole blood for scientific research in accordance with the Declaration of Helsinki. The project was approved

by the Central Denmark Regional Ethics Committee (no. M-2016-151-16).

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website.

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