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ORIGINAL ARTICLE

Meta-Analysis Confirms the *LCE3C_LCE3B* Deletion as a Risk Factor for Psoriasis in Several Ethnic Groups and Finds Interaction with *HLA-Cw6*

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A multicenter meta-analysis including data from 9,389 psoriasis patients and 9,477 control subjects was performed to investigate the contribution of the deletion of genes *LCE3C* and *LCE3B*, involved in skin barrier defense, to psoriasis susceptibility in different populations. The study confirms that the deletion of *LCE3C* and *LCE3B* is a common genetic factor for susceptibility to psoriasis in the European populations ($OR_{Overall} = 1.21$ (1.15–1.27)), and for the first time directly demonstrates the deletion's association with psoriasis in the Chinese (OR = 1.27 (1.16–1.34)) and Mongolian (OR = 2.08 (1.44–2.99)) populations. The analysis of the *HLA-Cw6* locus showed significant differences in the epistatic interaction with the *LCE3C* and *LCE3B* deletion in at least some European populations, indicating epistatic effects between these two major genetic contributors to psoriasis. The study highlights the value of examining genetic risk factors in multiple populations to identify genetic interactions, and indicates the need of further studies to understand the interaction of the skin barrier and the immune system in susceptibility to psoriasis.

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INTRODUCTION

Psoriasis is a common chronic inflammatory disease of the skin with a variable worldwide prevalence, being common in European descent individuals and less frequent in Asian ancestry populations (Bowcock, 2005). To date, several loci have been underlined as psoriasis risk susceptibility factors, with *PSORS1*, a major histocompatibility complex class I region on chromosome 6p21, being the locus with the largest effect identified to

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Abbreviations: LCE, *late cornified envelope;* LCE3C_LCE3B-del, *deletion of* LCE3C *and* LCE3B *genes; OR, odds ratio; SNP, single-nucleotide polymorphism*

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date (Nestle *et al.*, 2009). Within *PSORS1*, the *HLA-Cw06* allele has been pinpointed as the risk variant that confers the strongest susceptibility to psoriasis (Nair *et al.*, 2006).

In a previous study we reported the association of the deletion of two late cornified envelope (*LCE*) genes, *LCE3C* and *LCE3B* (*LCE3C_LCE3B-del*), with psoriasis in 1,426 unrelated psoriatic patients and 1,406 controls from four populations of European ancestry. The *LCE3C_LCE3B-del* involves a 32.2-kb deletion, removing genes *LCE3C* and *LCE3B* of the *LCE* cluster, which is part of the epidermal differentiation complex on chromosome 1q21.3. Association with rs4112788, a tag single-nucleotide polymorphism (SNP) for the biallelic *LCE3C_LCE3B-del* copy number variant, located 584 nucleotides downstream of *LCE3D*, was also found. Interaction analysis showed epistatic effects between the *LCE3C_LCE3B-del* and *HLA-Cw06* allele only in the Dutch population (de Cid *et al.*, 2009). At the time of publication, an independent genome-wide association scan in a Chinese cohort also identified association of rs4112788 with the disease, indicating a major role of the *LCE* locus in psoriasis susceptibility (Zhang *et al.*, 2009). Furthermore, this locus was replicated in a German case–control study of psoriasis vulgaris (Hüffmeier *et al.*, 2010a) and in a Spanish case–control study of chronic plaque-type psoriasis vulgaris (Coto *et al.*, 2010). Since these initial studies in psoriasis, the *LCE3C_LCE3B* locus has been evaluated in other and psoriasis-related phenotypes. Hüffmeier *et al.* (2010b) found no association of this locus with susceptibility to psoriatic arthritis in the German samples, whereas association with this phenotype has been detected in the British and Irish (Bowes *et al.*, 2010) and in the Spanish (Docampo *et al.*, 2010) patients. Finally, Bergboer *et al.* (2010) have found negative association of the *LCE3C_LCE3B* locus with atopic dermatitis.

The aim of this meta-analysis with individual patient data was to further investigate the contribution of *LCE3C_LCE3B-del* to psoriasis susceptibility and its possible interaction with the *PSORS1* locus. In all, 13 cohorts from 12 populations, 9 of European ancestry (Finland, France, Germany, Ireland, Italy, Spain, The Netherlands, United Kingdom, and United States (US-California (US-CA) and US-Michigan (US-MI)), and 3 of Asiatic origin (China, Mongolia, and Japan), were included in the study (see Supplementary Methods online for sample description). Overall, 9,389 psoriasis cases and 9,477 control samples were analyzed for the association of *LCE3C_LCE3B-del* with psoriasis. Association of rs4112788 was also investigated in 11 of the 13 data sets included. A possible relationship between *PSORS1* and *LCE3C_LCE3B-del* and its tag SNP was assessed through interaction analysis using directly typed *HLA-Cw06* when available, or rs130076, a SNP in linkage disequilibrium with it (Asumalahti *et al.*, 2002).

RESULTS AND DISCUSSION

Association analyses of the genotyping data confirmed that the deletion of both *LCE3C* and *LCE3B* genes is a susceptibility determinant for psoriasis in the European ancestry populations, with a significantly higher frequency of the *LCE3C_LCE3B-del* allele in psoriatic patients compared with control individuals ($OR_{Overall} = 1.21$ (1.15–1.27), $P_{Overall} = 4.58 \times 10^{-13}$; Table 1). As no significant evidence of heterogeneity between European ancestry populations was observed, a combined odds ratio (OR) was calculated under a population fixed effects model. In addition, the estimation of an overall OR under a population random effects model—which better accommodates potential heterogeneity across populations of the genetic effect estimates because of genuine differences and/or different biases—was practically identical, which is a further indication of absence of significant heterogeneity (Lau *et al.*, 1997; Ioannidis *et al.*, 2007) (Table 1 and Figure 1). At the genotype level, the analysis suggests a potential dosage effect with genotypes having two copies of genes *LCE3C* and *LCE3B* being a protective factor against the development of the disease in the European ancestry populations ($OR_{Overall} = 1.20$ (1.15–1.28), $P_{Overall} = 1.42 \times 10^{-13}$; Supplementary Table S1 online).

Genotyping of Asian population samples for the *LCE3C_LCE3B-del* confirmed that the strong genetic association with SNPs at the *LCE3* genes detected in the Chinese population (Zhang *et al.*, 2009) is because of the presence of the deletion of *LCE3C* and *LCE3B*. Genotyping for *LCE3C_LCE3B-del* in the other Asian populations further confirmed its presence in these populations. The deletion has the same sequence structure as that found in Caucasian populations. The detection of significant heterogeneity among the Asiatic population for *LCE3C_LCE3B-del* allelic frequencies impeded the estimation of the overall association of *LCE3C_LCE3B-del* with the psoriatic phenotype in Asian ancestry populations. The deletion is strongly associated with psoriasis in the Chinese and Mongolian populations with regard to both the allelic ($OR = 1.27$ (1.16–1.34), $P = 1.70 \times 10^{-07}$; $OR = 2.08$ (1.44–2.99), $P = 8.16 \times 10^{-05}$, respectively) and the genotype level ($OR = 1.28$ (1.16–1.41), $P = 1.41 \times 10^{-07}$; $OR = 2.04$ (1.41–2.94), $P = 9.38 \times 10^{-05}$, respectively) (Supplementary Table S1 online). In the Japanese population, however, the higher frequency of the deleted allele among the psoriatic individuals compared with controls did not reach the level of significance ($P = 0.063$; Table 1).

An analysis of rs4112788 showed association of allele C with the disease in the European ancestry populations ($OR_{Overall} = 1.21$ (1.15–1.27), $P_{Overall} = 1.42 \times 10^{-12}$; Table 1 and Figure 1) as well as in the Chinese population ($OR = 1.34$ (1.21–1.46), $P = 6.42 \times 10^{-10}$; Table 1). At the genotype level, a statistically significant higher risk for psoriasis was observed in individuals homozygous for the C allele in populations with European ancestry ($OR_{Overall} = 1.20$ (1.15–1.27), $P_{Overall} = 1.81 \times 10^{-12}$) as well as in China ($OR_{Overall} = 1.35$ (1.22–1.47), $P_{Overall} = 3.62 \times 10^{-12}$; Supplementary Table S2 online). The high coefficient of determination measure (r^2) (over 0.85 in all populations) indicates that rs4112788 is a close proxy of the *LCE3C_LCE3B-del* allele in the Chinese population also (Table 1). This is the first direct indication that the strong association of psoriasis with rs4112788, detected in the initial analysis in Chinese samples (Zhang *et al.*, 2009), is also associated with the *LCE3C_LCE3B-del* allele.

Interestingly, we observed a significant negative correlation between the frequency of *LCE3C_LCE3B-del* among controls and the corresponding OR for psoriasis for the eight populations from Europe examined—the more common the risk allele, the smaller its effect on psoriasis risk (Supplementary Figure S1 online). Allele frequency, and the correlated effect strength,

Table 1. Association of *LCE3C_LCE3B-del* and its tag SNP rs4112788 with psoriasis in individuals of European and Asian ancestry

		***LCE3C_LCE3B* CNV**					**rs4112788**					
Data set	**Status**	***LCE3C_LCE3B*-del**	**Intact**	**HWE**	**OR (95% CI) (del vs. intact)**	***P*-value**	**Allele C**	**Allele T**	**HWE**	**OR (95% CI) (C vs. T)**	***P*-value**	r^2
Spain	Control	420 (55.0)	344 (45.0)	0.10	1.49 (1.15–1.93)	0.00284	427 (55.9)	337 (44.1)	0.67	1.57 (1.21–2.05)	0.00079	0.92
	Psor	227 (64.5)	125 (35.5)	0.02			233 (66.6)	117 (33.4)	0.09			
Italy	Control	516 (57.3)	384 (42.7)	0.03	1.30 (1.08–1.58)	0.00603	510 (57.2)	382 (42.8)	0.08	1.39 (1.15–1.68)	0.00079	0.93
	Psor	573 (63.7)	327 (36.3)	0.48			583 (64.9)	315 (35.1)	1.00			
France	Control	211 (64.3)	117 (35.7)	0.08	1.26 (0.90–1.77)	0.1767	216 (65.1)	116 (34.9)	0.06	1.29 (0.99–1.95)	0.1405	0.96
	Psor	196 (69.5)	86 (30.5)	1.00			202 (70.6)	84 (29.4)	1.00			
The Netherlands	Control	334 (59.6)	226 (40.4)	0.54	1.50 (1.14–1.96)	0.00329	333 (59.9)	223 (40.1)	0.62	1.54 (1.18–2.02)	0.00155	0.99
	Psor	281 (68.9)	127 (31.1)	0.63			278 (68.8)	126 (31.2)	0.74			
Germany	Control	1,215 (64.9)	657 (35.1)	0.67	1.31 (1.15–1.48)	3.03e-05	1,151 (64.7)	627 (35.3)	0.56	1.22 (1.07–1.38)	0.00229	0.94
	Psor	1,899 (70.8)	785 (29.2)	0.43			1,799 (69.1)	803 (30.9)	0.47			
UK	Control	1,370 (67.1)	672 (32.9)	0.94	1.16 (1.01–1.33)	0.0306	1,303 (66.4)	659 (33.4)	0.57	1.12 (0.98–1.28)	0.0872	0.86
	Psor	1,323 (70.3)	559 (29.7)	0.10			1,390 (68.9)	626 (31.1)	0.30			
Ireland	Control	1,311 (69.2)	583 (30.8)	0.17	1.07 (0.89–1.29)	0.459	1,335 (68.8)	605 (31.2)	0.33	1.18 (0.99–1.41)	0.0695	0.94
	Psor	554 (70.7)	230 (29.3)	0.90			624 (72.2)	240 (27.8)	0.47			
Finland	Control	436 (65.1)	234 (34.9)	0.05	1.37 (1.01–1.87)	0.046	433 (65.6)	227 (34.3)	0.09	1.37 (1.00–1.88)	0.0512	0.98
	Psor	194 (71.9)	76 (28.1)	0.39			188 (72.3)	72 (27.7)	0.19			
US-CA	Control	378 (64.3)	210 (37.7)	0.04	1.36 (1.11–1.68)	0.00378	379 (64.0)	213 (36.0)	0.13	1.37 (1.11–1.69)	0.00339	0.95
	Psor	847 (71.1)	345 (28.9)	0.09			835 (70.9)	343 (29.1)	0.06			
US-MI	Control	2,478 (64.8)	1,348 (35.2)	0.52	1.09 (1.00–1.20)	0.0584	2,472 (65.0)	1332 (35.0)	0.45	1.10 (1.00–1.20)	0.0515	0.98
	Psor	2,835 (66.8)	1,411 (33.2)	0.33			2,828 (67.0)	1,390 (33.0)	0.46			
Overall[1]	Control	6,028 (64.2)	3,364 (35.8)	—	1.21 (1.15–1.27)[2]	4.58e–13[2]	6,087 (64.2)	3,389 (35.8)	—	1.21 (1.15–1.27)[2]	1.42e–12[2]	—
	Psor	5,902 (69.6)	2,580 (30.4)	—	1.21 (1.15–1.28)[3]	1.47e–12[3]	6,132 (69.2)	2,726 (30.8)	—	1.21 (1.15–1.28)[3]	1.44e–12[3]	
China	Control	2,174 (57.3)	1,620 (42.7)	0.95	1.27 (1.16–1.34)	1.70e-07	2,173 (57.6)	1,601 (42.4)	1.0	1.34 (1.21–1.46)	6.42e–10	0.91
	Psor	2,518 (63.1)	1,472 (36.9)	0.21			2,543 (64.4)	1,403 (35.6)	0.01			
Japan	Control	631 (58.8)	443 (41.2)	0.79	1.17 (0.99–1.40)	0.0638	ND	ND	—	—	—	—
	Psor	689 (62.6)	411 (37.4)	1.00								
Mongolia	Control	166 (49.4)	170 (50.6)	0.36	2.08 (1.44–2.99)	8.16e-05	ND	ND	—	—	—	—
	Psor	134 (67.0)	66 (33)	0.82								

Abbreviations: CI, confidence interval; CNV, copy number variant; HWE, Hardy–Weinberg equilibrium; ND, no data available; OR, odds ratio; SNP, single-nucleotide polymorphism; US-CA, US-California data set; US-MI: US-Michigan data set.
[1]Overall analyses for the European ancestry populations are computed according to a logistic model in which population was introduced as a confounding variable (based on a [2]fixed effects model and a [3]random effects model) after no significant evidence of heterogeneity was detected according to the Woolf test on homogeneity of odds ratios (*P*=0.0763 for *LCE3C_LCE3B* CNV; *P*=0.0553 for rs4112788). Overall values for the Asian ancestry populations are not presented as the Woolf test on homogeneity of odds ratios showed statistical significant heterogeneity among them (*P*=0.02091). Predictive performance of allele C with *LCE3C_LCE3B-del* is presented for each population using the coefficient of determination measure (r^2).

appears to follow an approximate north–south gradient pattern. Even though this observation could be because of sampling error, and additional European populations would need to be studied, a genuine significance of this phenomenon on the genetic predisposition to psoriasis cannot be ruled out.

Direct typing of *HLA-Cw06* in The Netherlands, Italy, Japan, Mongolia, and the US samples allowed the estimation of a potential interaction between the *LCE3C_LCE3B* deletion, or its tag SNP rs4112788, with *PSORS1* locus. Apart from the already known interaction observed in the Dutch population alone, evidence for interaction was also observed in the US-MI data set, but not in the Italian sample. The existence of heterogeneity among the cohorts with European ancestry prevented the analysis of the interaction in those cohorts as a whole. Evidence of interaction between either *LCE3C_LCE3B-del* or *rs4112788* with *HLA-Cw06* was not observed in the Japanese and Mongolian data sets (Table 2).

In the remaining populations, interaction with *PSORS1* was assessed through its proxy marker rs130076. No evidence for interaction was seen with *LCE3C_LCE3B-del* or its tag SNP in any of the populations interrogated (Supplementary Table S3 online). To investigate whether the association of rs130076 with psoriasis (Supplementary Table S4 online) is independent or secondary to *HLA-Cw06*, the effect of this SNP was analyzed in a stratified analysis that defined strata by carriage of *HLA-Cw06* in the Italian data set (as the Italian was the

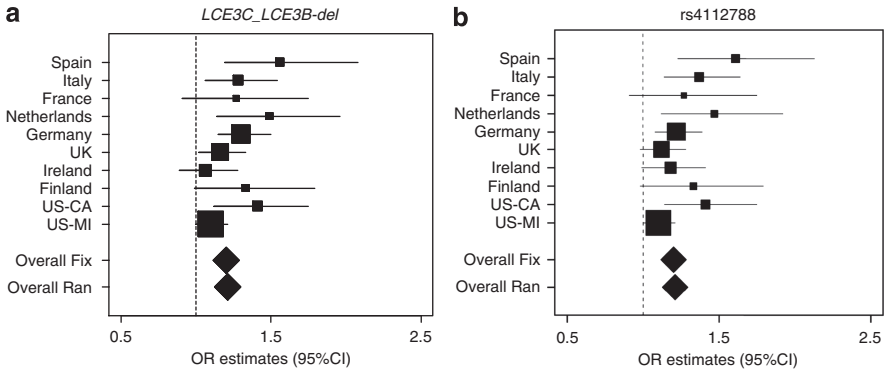

Figure 1. Meta-analysis of *LCE3C_LCE3B-del* and rs4112788 for association with psoriasis across populations of European ancestry. Panel **a** shows the data for *LCE3C_LCE3B-del* and panel **b** for rs4112788. Squares show the point estimate of the odds ratio (OR) and its 95% confidence intervals (95% CIs) with regard to genotype frequencies. Diamonds show the summary effect by fixed (Overall Fix) and random (Overall Ran) effects model. Different square sizes represent different weights of each population.

Table 2. Genetic interaction analysis between *HLACw06* in *PSORS1* and *LCE3C_LCE3B-del* and its rs4112788 tag SNP

	***HLA-Cw06*[1]**				**Epistasis[2]**					
	Positive vs. negative				**rs4112788-*HLA-Cw06***			***LCE3C_LCE3B- HLA-Cw06***		
Data set	**OR**	**95% CI**	***P*-value**	**Group**	**OR**	**95% CI**	***P*-value**	**OR**	**95% CI**	***P*-value**
The Netherlands	3.45	2.27–5.25	2.974e-09	+	2.58	1.46–4.57	0.0160	2.60	1.47–459	0.0180
				–	1.15	0.83–1.60		1.17	0.84–1.63	
Italy	2.5	1.86–3.36	4.994e-10	+	1.22	0.45–1.75	0.57897	1.14	0.80–1.62	0.5445
				–	1.38	1.09–1.76		1.30	1.03–1.64	
US-MI	3.69	3.19–4.27	1.063e-75	+	1.44	1.19–1.75	0.00028	1.44	1.19–1.75	0.00027
				–	0.95	0.85–1.06		0.95	0.85–1.06	
Japan	9.25	3.94–21.7	6.854e-11	+	ND			1.09	0.23–5.08	0.9266
				–	ND			1.17	0.98–1.39	
Mongolia	34.39	16.48–1.7	1.902e-31	+	ND			1.46	0.82–2.59	0.1286
				–	ND			3.67	1.30–10.37	

Abbreviations: CI, confidence interval; ND, no data; OR, odds ratio; SNP, single-nucleotide polymorphism; US-MI, US-Michigan data set.
[1]OR and 95% CI for psoriasis of directly typed *HLA-Cw06* was analyzed using the carrier status definition for *Cw06* allele.
[2]Epistasis analysis performed by logistic regression models that included an interaction term (rs4112788-*HLA-Cw06* or *LCE3C_LCE3B*-del-*HLA-Cw06*); *P*-values are derived from the log-likelihood ratio test between the model including both additive effects plus the interaction term against the model that only includes additive effects. Overall values for the European and Asian ancestry populations are not presented, as significant heterogeneity based on allelic frequencies was detected by population according to the Woolf test on homogeneity of ORs (*P*=0.0045 and *P*=0.0012, respectively).

only population in which both rs130076 and *HLA-Cw06* were genotyped). In the subset of samples that does not contain an *HLA-Cw06* allele, rs130076 was no longer significantly associated with psoriasis (OR = 4.64 (2.74–7.84), $P = 3.61 \times 10^{-09}$ in *HLA-Cw06*-positive samples vs. OR = 1.05 (0.73–1.50), $P = 0.7938$ in *HLA-Cw06*-negative samples). This suggests that the association of rs130076 is dependent on *HLA-Cw06*, but also that this SNP is an imperfect surrogate for *HLA-Cw06*, at least in the Italian population. Therefore, although it is valid to perform interaction analyses using rs130076, these analyses will likely have less power than those that use directly typed *HLA-Cw6*. Hence, it may not be coincidental that significant interaction was detected in two of the five data sets with *HLA-Cw06* typing, but in none of the eight data sets with rs130076 typing. The existence of a potential epistasis found only in the Dutch and US-MI data sets, but in none of the remaining data sets in which *HLA-Cw06* was typed, might be because of population-specific effects, different genetic backgrounds, or varying environmental exposures among data sets. The fact that no interaction was observed between *LCE3C_LCE3B-del* and *HLA-Cw06* in the Chinese data set is probably because of the fact that despite *HLA-Cw6* being a major risk allele for psoriasis in the Chinese population, it does not explain by itself the full linkage evidence of the *PSORS1* locus in that population (Fan *et al.*, 2008).

In summary, we have confirmed that the deletion of genes *LCE3C* and *LCE3B* is a common genetic factor for susceptibility to psoriasis in European populations, and for the first time directly demonstrated the deletion's association with psoriasis in some Asian groups. Interestingly, we detected significant differences in the epistatic interaction of the deletion with *HLA-Cw6*, with a positive interaction in the

Dutch and US-MI samples but no interaction with other European cohorts. This study highlights the value of examining genetic risk factors in multiple populations, and suggests that further studies in experimental models of disease are needed to understand the interaction of the skin barrier and the immune system in susceptibility to psoriasis.

MATERIALS AND METHODS

Genotyping

Typing of the *LCE3C_LCE3B-del* copy number variant was performed by direct PCR using a four-primer or three-primer assay as previously described (de Cid *et al.*, 2009), allowing the simultaneous detection of intact and deleted alleles. Genotyping rates for *LCE3C_LCE3B-del* ranged from 92.5 to 100% in all European ancestry populations and from 97.3 to 100% in Asian populations. With regard to rs4112788, genotyping rates ranged from 93.7 to 99.6% in the European ancestry populations, whereas it reached 99.2% in the Chinese population. SNP assays in Spain, Netherlands, Italy, and US-CA were genotyped as previously described (de Cid *et al.*, 2009). In the Ireland data set, genotyping of SNPs was performed using competitive allele-specific PCR at Kbiosciences (Hoddesdon, Herts, UK), and in Finnish data set using matrix-assisted laser desorption/ionization time-of-light mass spectrometry (Sequenom, San Diego, CA). In the remaining populations, genotyping of SNPs was conducted using TaqMan assays (Applied Biosystems, Foster City, CA). HLA allele discrimination in sample collections from The Netherlands and Italy were performed as described (de Cid *et al.*, 2009). In the Japanese and Mongolian cohorts, HLA typing was conducted with LABType SSO typing test (One Lambda, Canoga Park, CA) and LABScan 100 flow analyzer. *HLA-Cw06* genotypes in the US-MI sample were determined by genotyping seven SNPs in exons 2 and 3 of the *HLA-C* gene, as previously described (Nair *et al.*, 2006).

Statistical analysis

LCE3C_LCE3B-del and SNP association analysis. Logistic regression models assessed the genetic effect of the *LCE3C_LCE3B-del* and SNPs on psoriasis risk. Calculations for genotype frequency differences were performed by regression analysis for co-dominant, dominant, recessive, and log-additive models. The best genetic model was selected using the Akaike information criteria. Heterogeneity among populations was assessed using the Woolf test that evaluates the homogeneity of ORs. Overall values were calculated when the homogeneity assumption among populations was plausible, and were adjusted by population according to a logistic model that introduces population as a confounding variable. Potential interaction between *LCE3C_LCE3B-del* or rs4112788 and *HLA-Cw06* or rs130076 was evaluated from the log-likelihood ratio test between a model that includes both the additive effect and the interaction term against a model that only includes additive effects.

CONFLICT OF INTEREST

Dr Henry W. Lim has served as a consultant for La Roche-Posay, Orfagen, and Clinuvel. These activities do not have an influence on the results presented in this paper. The other authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

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