The HLA-DQB1*03:01 Is Associated with Bullous Pemphigoid in the Han Chinese Population

Journal of Investigative Dermatology (2018) **138,** 1874–1877; doi:10.1016/j.jid.2018.02.021

TO THE EDITOR

Bullous pemphigoid (BP) is a potentially fatal subepidermal blistering autoimmune disease that characteristically affects elderly patients who present with large tense bullae on the entire skin and frequently on the extremities (Nousari et al., 1999; Sami et al., 2004). Subepidermal blisters with inflammation are the hallmark of BP histology. Direct immunofluorescence of perilesional skin shows linear deposits of the C3 and IgG. Two hemidesmosomal antigens have been identified, one protein of approximately 230 kDa associated with the intracellular plaque (BPAG1, also known as BP230) (Stanley et al., 1981), and the other protein of approximately 180 kDa associated with transmembrane glycoprotein (BPAG2, also known as BP180) (Diaz et al., 1990). Antibodies against both BPAG1 and BPAG2, as measured by ELISA, have been used for the diagnosis of BP (Roussel et al., 2011).

Although the heritability has not been well understood, genetic studies have revealed that persons with certain HLA alleles have higher risk of specific autoimmune blistering disease than persons without these alleles. Currently, the genetic data of BP are sparse and inconsistent. Various association results were reported for BP in different studies, such as the association of DQB1*03:01 with BP in Caucasians (Delgado et al., 1996) and Iranians (Esmaili et al., 2013), and the association of DQB1*03:02/DRB1*11:01/DRB1*04:03 in Japanese BP (Okazaki et al., 2000). Associations between HLA-DQB1*0301 have been reported frequently for the clinical variants of BP, including mucous membrane pemphigoid of oral and ocular mucosae (Chan et al., 1997; Setterfield et al., 2001). In a recent genetic investigation of 25 Chinese BP patients and 57 controls (Gao et al., 2002), only lower frequencies of DRB1*08 (DR8) and DRB1*08/DQB1*06 (DR8/DQ6) haplotypes were observed in BP patients compared to controls ($P < 0.05$), which suggested ethnic differences may influence the genetic susceptibility to BP. Furthermore, other studies have reported that *DQB1*03:01* was restricted to a different phenotype, involving the response of $CD4^+$ T cells to BPAG2 (Büdinger et al., 1998), or was only associated with male BP patients (Banfield et al., 1998). Aiming to discover genetic risk factors for BP and to fine-map the associations within the extended major histocompatibility complex region, we conducted a genetic association analysis study by using a next-generation sequence-based HLA typing method in a sample of 575 pemphigoid patients and 976 healthy controls of Chinese descent.

All of the BP cases were recruited from Shandong Provincial Institute of Dermatology and Venereology from 2006 to the present. The diagnosis of BP was based on the combination of clinical data and a predominantly subepidermal blistering picture, the linear deposition of IgG or C3 along the base membrane zone by direct immunofluorescence microscopy, and/or serological ELISA tests using commercially available BP180- and BP230-specific serological kits (Medical & Biological Laboratories, Nagoya, Japan). Direct immunofluorescence microscopy of salt split skin or seronegative results of circulating autoantibodies against type VII collagen by ELISA (Medical & Biological Laboratories) were employed to exclude the diagnosis of epidermolysis bullosa acquisita.

Basic characteristics of all 575 BP patients are shown in Table 1. There were 327 (56.9%) male and 248 (43.1%) female patients. Mean age at onset of BP was 69 years, ranging from 0 to 97 years without gender bias. Three infant BP patients, who were less than 1 year old and reported as case series previously (Yang et al., 2016), were also included in the study. The study was approved by the Institutional Review Board of Shandong Provincial Institute of Dermatology and Venereology and all the subjects or their parents gave written informed consent.

Direct immunofluorescence microscopy of perilesional skin biopsies showed linear deposits of C3 at the base membrane zone in all BP patients (100%), while 379 BP patients (67.2%) showed IgG deposits or weak deposits. In addition, 352 BP patients and 188 BP patients showed IgA (62.4%) and IgM deposits (33.3%) or weak deposits at the base membrane zone, respectively. Eight combined deposit patterns were identified in BP patients. The three most common observed patterns were, $C3^+$-IgG^+-IgA^+-IgM^+ (29.8%), $C3^+$-IgG^+-IgA^+-IgM^- (27.3%), and $C3^+$-IgG^--IgA^--IgM^- (26.6%), which together accounted for 83.7% of all BP patients. The rarest pattern was $C3^+$-IgG^--IgA^--IgM^+, which was found in only five BP patients (0.9%).

The positive rates of anti-BP180 and anti-BP230 were also assessed by ELISA tests. As expected, more BP patients showed anti-BP180–positive (94.7%) than anti-BP230–positive (54.4%). Dual-positivity for both antibodies occurred in 241 BP patients (49.1%), while 250 BP patients were single-positive (224 for anti-BP180–positive

Abbreviations: BP, bullous pemphigoid; OR, odds ratio

Accepted manuscript published online 27 February 2018; corrected proof published online 19 April 2018

Table 1. Basic characteristics and histological features of all the bullous pemphigoid patients and controls

Category	Patients	Controls
Gender, n (%)		
Male	327 (56.9)	596 (61.1)
Female	248 (43.1)	380 (38.9)
Total, n	575	976
Age, y, mean (range)		
Male	68.9 (0–93)	61.9 (23–82)
Female	69.0 (0–97)	61.4 (22–91)
All	69.0 (0–97)	61.7 (22–91)
Antigen, n (%)		
BP180-positive	465 (94.7)	NA
BP230-positive	267 (54.4)	NA
BP180-positive and BP230-negative	224 (45.6)	NA
BP230-positive and BP180-negative	26 (5.3)	NA
BP180- and BP230-positive	241 (49.1)	NA
Total, n	491	NA
Histology, n (%)		
IgA	352 (62.4)	NA
IgM	188 (33.3)	NA
IgG	379 (67.2)	NA
C3	564 (100)	NA
Total, n	564	NA
Combination of antibodies, n (%)		
C3-IgG-IgA-IgM	168 (29.8)	NA
C3-IgG-IgA	154 (27.3)	NA
C3-IgG-IgM	7 (1.2)	NA
C3-IgA-IgM	8 (1.4)	NA
C3-IgG	50 (8.9)	NA
C3-IgA	22 (3.9)	NA
C3-IgM	5 (0.9)	NA
C3	150 (26.6)	NA
Total	564	NA

Abbreviation: NA, not available.

alone and 26 for anti-BP230–positive alone).

To investigate the associations within the major histocompatibility complex region, we conducted high coverage next-generation sequence-based HLA typing analysis on five classical HLA alleles—*HLA-A*, *-B*, *-C*, *-DRB1*, and *-DQB1*. Consequently, the whole genomic region of class I major histocompatibility complex alleles and the polymorphic region (exon 2, 3, and 4) of class II major histocompatibility complex alleles were amplified and then sequenced by using illumina Miseq platform (Illumina Inc, San Diego, CA). A mean coverage of 622.8x for HLA across all the samples was identified (511.3x for *-A*, 472.1x for *-B*, 434.9 for *-C*, 736.2x for *-DRB1*, and 885.8x for *-DQB1*). The high-resolution classical HLA alleles were subsequently called with default settings by the software NGSengine (GenDX, Utrecht, The Netherlands). After stringent quality control (three patients failed this step), 572 BP patients and 976 healthy controls were involved in the subsequent analysis.

Classical HLA alleles were defined as a series of binary markers (present/absent). We applied the logistic regression framework to test each of these binary markers for association. The most significant risk association for BP was demonstrated to be *DQB1*03:01* ($P = 1.27 \times 10^{-7}$; odds ratio [OR] = 1.582; 95% confidence interval = 1.334–1.875; Table 2). *DQB1*03:03* ($P = 6.11 \times 10^{-7}$; OR = 0.552; 95% confidence interval = 0.437–0.697) was identified as the most significant protective allele for BP, while *DQB1*06:01* ($P = 1.18 \times 10^{-5}$; OR = 0.478; 95% confidence interval = 0.344–0.665) was identified as a second significant protective allele. These three classical alleles were independent from each other, and conditioning on the effect of them could eliminate all the other associations. The significance level was estimated at 1.86×10^{-4}, with 269 classical alleles were involved in the association analysis.

Associations tests first stratified by gender were reported in a previous publication (Banfield et al., 1998), but no significant results were observed ($P > 0.05$ for all the three markers). We then stratified the BP patients into BPAG1-positive and BPAG2-positive groups and conducted association analysis comparing them to the healthy controls. The two protective alleles, *DQB1*03:03* and *DQB1*06:01*, showed similar ORs and *P* values for both antibody groups. However, *DQB1*03:01* showed a significant difference between the BPAG1 and BPAG2 groups. Although both groups were significantly associated, the risk effect for the BPAG2 group ($P = 7.99 \times 10^{-8}$; OR = 1.638) was stronger than the BPAG1 group ($P = 5.65 \times 10^{-3}$; OR = 1.371).

Finally, we evaluated the effectiveness of *DQB1*03:01* for risk prediction. *DQB1*03:01* was presented in 49.65% of the patients (284 of 572), but only 35.25% of the healthy controls (344 of 976). For BP, it suggests that the presence of *DQB1*03:01* has a sensitivity of 49.65% and a specificity of 64.75% as a risk predictor, with an area under the curve of 0.573 for disease prediction. On the basis of the estimated prevalence of the BP (2/100,000), we calculated the predictive value of the classical HLA allele for BP. *DQB1*03:01* would have a negative predictive value of 99.99%, which meant that nearly all the negative samples represented true-negative samples, but a positive predictive value of 0.0028% was caused by the rare prevalence of disease.

Although the nature of the trigger remains unresolved in most of the autoimmune blistering diseases, a strong association between HLA and the particular autoimmune blistering disease has been established for several dozens of years. In this study, we confirmed *DQB1*03:01* as the only significant risk association for BP in the Chinese population, while *DQB1*03:03* and

Table 2. Significant association results and stratified analysis by gender and antibody

Allele	F_A	F_U	*P*-Value	OR	L95	U95
HLA_DQB1_0301	0.285	0.2	1.27E-07	1.582	1.334	1.875
HLA_DQB1_0303	0.095	0.16	6.11E-07	0.552	0.437	0.697
HLA_DQB1_0601	0.043	0.085	1.18E-05	0.478	0.344	0.665

Allele	F_Male	F_Female	*P*-Value	OR	L95	U95
Stratified by gender						
HLA_DQB1_0301	0.283	0.287	8.69E-01	0.978	0.751	1.274
HLA_DQB1_0303	0.091	0.101	5.38E-01	0.879	0.583	1.325
HLA_DQB1_0601	0.045	0.04	7.27E-01	1.112	0.613	2.017

Allele	F_BPAG2	F_BPAG1	F_U	*P*-Value_1	OR_1	L95_1	U95_1	*P*-Value_2	OR_2	L95_2	U95_2
Stratified by antibody											
HLA_DQB1_0301	0.293	0.256	0.2	7.99E-08	1.638	1.368	1.961	5.65E-03	1.371	1.096	1.716
HLA_DQB1_0303	0.097	0.107	0.16	8.10E-06	0.565	0.44	0.726	2.45E-03	0.631	0.467	0.852
HLA_DQB1_0601	0.045	0.043	0.085	1.53E-04	0.508	0.358	0.721	1.26E-03	0.486	0.311	0.76

Abbreviations: F_A, allele frequency in affected cases; F_BPAG2, allele frequency in BPAG2-positive patients; F_BPAG1, allele frequency in BPAG1-positive patients; F_Female, allele frequency in female patients; F_Male, allele frequency in male patients; F_U, allele frequency in unaffected controls; OR, odds ratio.

*DQB1*06:01* were demonstrated to be significant protective associations. The risk effect of *DQB1*03:01* was much weaker than that found in the Caucasian mucous membrane pemphigoid patients, in which 75.8% patients carried the risk allele (Setterfield et al., 2001). After we stratified the patients into BPAG1-positive and BPAG2-positive groups, we observed increased allele frequency for *DQB1*03:01* in the BPAG2-positive group. Although the *DQB1*03:01* association was still nominally significant in the BPAG1-positive group, these findings would suggest that *DQB1*03:01* is more restricted to BPAG2 antigen presenting.

*DQB1*03:01* has been reported to be associated with multiple diseases, such as cutaneous melanoma (Lee et al., 1994) and familial generalized vitiligo (Fain et al., 2006). Although its precise effect in the pathogenesis is unclear, it has been hypothesized that the HLA allele may play a pivotal role in the recognition of antigenic peptides by T cells (Hassan et al., 2011).

In conclusion, this study represents, to our knowledge, the largest genetic study of BP, as well as the first HLA analysis by using next-generation sequence-based HLA typing methods in BP. Through this investigation, the associations of HLA-DQB1*03:01, -DQB1*03:03, and DQB1*06:01 have been identified in Chinese Han BP patients, which advances our understanding of the genetics of BP susceptibility and offers molecular insight into the pathophysiological mechanisms underlying BP in the Chinese Han population.

CONFLICT OF INTEREST

The authors state no conflict of interest.

ACKNOWLEDGMENTS

We thank all of the participants involved in this study. This work was funded by grants from the National Natural Science Foundation of China (816201008025, 81472868, 81472869, 81502736), the Natural Science Foundation of Shandong Province (JQ201616 , 2014ZRC03128), the Key Research and Development Program of Shandong Province (2016ZDJS07A06), the Innovation Project of Shandong Academy of Medical Sciences, and the Shandong Provincial Advanced Taishan Scholar Construction Project.

***Yonghu Sun*[1,2,3,4], *Hong Liu*[1,2,3,4,*], *Zhenzhen Wang*[1,3,4], *Xi'an Fu*[1,3,4], *Chuan Wang*[1,3,4], *Zihao Mi*[1,3,4], *Lele Sun*[1,3,4], *Fangfang Bao*[1,3,4], *Gongqi Yu*[1,3,4], *Guizhi Zhou*[1,2] and *Furen Zhang*[1,2,3,4,*]**

[1]*Shandong Provincial Institute of Dermatology and Venereology, Shandong Academy of Medical Sciences, Jinan, Shandong, China;* [2]*Shandong Provincial Hospital for Skin Diseases, Shandong University, Jinan, Shandong, China;* [3]*Shandong Provincial Key Lab for Dermatovenereology, Jinan, Shandong, China; and* [4]*National Clinical Key Project of Dermatology and Venereology, Jinan, Shandong, China*

[*]*Corresponding author e-mail: zhangfuren@hotmail.com (Furen Zhang) or hongyue2519@hotmail.com (Hong Liu)*

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Facial Wrinkles in Europeans: A Genome-Wide Association Study

Journal of Investigative Dermatology (2018) **138,** 1877–1880; doi:10.1016/j.jid.2017.12.037

TO THE EDITOR

Wrinkles are among the most notable components of skin aging and are influenced by many different risk factors (Hamer et al., 2017). Although wrinkle variation has been shown to be a heritable trait, (55%) (Gunn et al., 2009), specific gene variants for wrinkles have not yet been identified. Previous studies have identified the *MC1R* gene as influencing skin photoaging and pigmented spots (Elfakir et al., 2010; Jacobs et al., 2015; Liu et al., 2016; Suppa et al., 2011), but its role in wrinkling is not clear. In this study, we performed the largest genome-wide association study (GWAS) for global facial wrinkles available to date in 3,513 participants from the Rotterdam Study (RS) using a digital wrinkle measure (Hamer et al., 2017) and sought to replicate the most suggestive associations in an independent dataset of 599 participants from the Leiden Longevity Study (LLS).

A detailed description of the methods is presented in the Supplementary Materials online. The RS is an ongoing Dutch prospective population-based cohort study of 14,926 participants aged 45 years or older (Hofman et al., 2015). This study includes 3,513 northwestern European participants for whom standardized facial photographs and quality-controlled genotype data were available. The RS has been approved by the Medical Ethics Committee of the Erasmus University Medical Center and by the Ministry of Health, Welfare and Sports of The Netherlands, which are implementing the Wet Bevolkingsonderzoek: ERGO (Population Studies Act: Rotterdam Study). All participants provided written informed consent to participate in the study. The LLS is a family-based study (Westendorp et al., 2009) that includes 599 participants in this study. In the RS, wrinkle area was digitally quantified as wrinkle area percentage of the face using semiautomated image analysis of high-resolution facial photographs. For wrinkle grading in the LLS, a 9-point photonumeric scale was used (Gunn et al., 2009). The study protocol was approved by the medical ethics committee of the Leiden University Medical Center, and all participants gave written informed consent. In the RS, DNA from whole blood was extracted following standard protocols, and quality controls were applied on markers and individuals (Hofman et al., 2015). Imputations were performed with 1000 Genomes (GIANT phase I version 3) as the reference panel (1000 Genomes Project Consortium et al., 2012). In total, 30,072,738 markers were genotyped/imputed. After quality controls, 9,009,554 autosomal single-nucleotide polymorphisms (SNPs) were available. In the LLS, imputation was performed similarly, and association testing was conducted using QT-assoc (Uh et al., 2015). The RS served as the discovery dataset. We performed linear regression using an additive model (SNP dosage data; Aulchenko et al., 2010) adjusting for age, sex, the first four genetic principal components, and two technical variables. These last two variables correct for possible variations in resolution and flash light of the facial photos (Hamer et al., 2017). For variations in resolution, a variable describing the batch number was used. For flash light variation, the in-person difference between skin lightness in the images and that taken by a spectrophotometer (CM-600d; Konica-Minolta, Osaka, Japan) on the cheek was used by calculating the residuals of these two lightness variables regressed on each other (Jacobs et al., 2015). We selected all SNPs with *P*-values less than 5×10^{-6} for the replication phase. We also performed a meta-analysis of the RS and LLS together for the top hits, as well as a genome-wide meta-analysis. Several sensitivity analyses (top SNP associations in men and women separately; with different facial wrinkling sites; possible interactions between SNPs and sex, body mass index, and smoking; and a univariate analysis excluding age and sex) and validation of previously published associations between SNPs and skin aging were performed (see Supplementary Materials).

In the RS, most participants were women (n = 2,045, 58.2%), and the

Abbreviations: GWAS, genome-wide association study; LD, linkage disequilibrium; LLS, Leiden Longevity Study; RS, Rotterdam Study; SNP, single nucleotide polymorphism

Accepted manuscript published online 16 March 2018; corrected proof published online 18 May 2018