HLA-DQB1*03:01 as a Biomarker for Genetic Susceptibility to Bullous Pemphigoid Induced by DPP-4 Inhibitors

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TO THE EDITOR

Dipeptidyl peptidase-4 inhibitor (DPP-4i) has been widely used to treat type 2 diabetes. DPP-4 inactivates incretins by catalyzing the cleavage of those proteins to inactive forms (Drucker, 2007). DPP-4i works by inhibiting the action of this enzyme and improves glycemic control (Aschner and Kipnes, 2006). DPP-4i has been known as a safe drug; however, an increased risk of bullous pemphigoid (BP) during DPP-4i exposure has been reported in diabetic patients administered DPP-4i (Béné et al., 2016).

BP is the most common autoimmune blistering disorder, and it is characterized by itchy edematous erythema and tense blisters on the whole body. It is mainly caused by autoantibodies to a major hemidesmosomal component at the dermal-epidermal junction of the skin, type XVII collagen (COL17 or BP180). The noncollagenous 16A (NC16A) domain of COL17 contains a major pathogenic epitope (Giudice et al., 1993). Although several factors have been reported as triggers of BP, the etiology of BP remains largely unknown.

The exact mechanism behind the association of DPP-4i exposure and BP has yet to be elucidated. Because several studies have reported an association between HLAs and drug-induced reactions (Chung et al., 2004; Wang et al., 2013), we examined HLA alleles in Japanese patients with BP who had been taking DPP-4i for type 2 diabetes for at least 3 months before BP onset (DPP-4i-BP). We recently reported that DPP-4i-BP tends to show a noninflammatory phenotype with few erythematous lesions, in sharp contrast to conventional BP unrelated to DPP-4i intake (Izumi et al., 2016). We encountered 30 patients with DPP-4i-BP in the last 3 years and found that most patients (21/30) showed the noninflammatory phenotype (Figure 1a). Based on the scores for erythema/urticaria in the bullous pemphigoid disease area index (BPDAI) (Murrell et al., 2012), DPP-4i-BP was clearly divided into two groups, inflammatory (BPDAI: erythema/urticaria $\geq$ 10) and noninflammatory (BPDAI: erythema/urticaria $<$ 10) (Figure 1b), and the clinical appearance of the patients with noninflammatory disease was distinct from that of those with conventional BP (Figure 1a). BPDAI scores for erosions/blisters showed no significant difference between DPP-4i-BP and conventional BP patients (Figure 1c). The antibody titers to full-length COL17 were similar between the two groups (Figure 1d), whereas those to the NC16A domain of COL17 were significantly lower in the noninflammatory DPP-4i-BP patients (Figure 1e). Histologically, eosinophil counts in the upper dermis of periblister lesions were significantly lower in noninflammatory DPP-4i-BP than in inflammatory DPP-4i-BP (Figure 1f). From these findings, we considered this unique noninflammatory subgroup to be distinct from inflammatory DPP-4i-BP and conventional BP, and this study focuses on this subgroup (Figure 1b red square [blue dots], and see Supplementary Tables S1 and S2 online). The collection of human samples was approved by the local ethics committee and the institutional review board of Hokkaido University and Keio University and by the research ethics committee of RIKEN. Written informed patient consent was obtained from the patients.

Surprisingly, 86% (18/21) of noninflammatory DPP-4i-BP patients in our sample carry HLA-DQB1*03:01 (Table 1). The frequencies of carriers of alleles HLA-DQB1*03:01, -DQA1*05:05, -DRB1*11:01, and -DRB1*12:01 were significantly higher, and those of carriers of alleles HLA-DQA1*01:03 and -DQB1*06:01 were significantly lower, in DPP-4i-BP than in Japanese general population control individuals (Table 1, and see Supplementary Tables S3–S9 online). We also compared the six HLA alleles in conventional BP patients with those in Japanese general population control individuals and found that none of those alleles was significantly different (Table 1). We next compared the six alleles in DPP-4i-BP with those in DPP-4i–tolerant patients with type 2 diabetes who were exposed to DPP-4i for at least 2 years (see Supplementary Table S10 online) and found that the frequencies of carriers of alleles HLA-DQB1*03:01 and -DRB1*12:01 were significantly higher in DPP-4i-BP (Table 1). These findings clearly show that the two alleles are significantly associated with DPP-4i-BP but not with conventional BP nor with type 2 diabetes. HLA-DQB1*03:01 was present in 19 (31%) of the 61 DPP-4i–tolerant control individuals, suggesting that this allele has 86% sensitivity and 69% specificity when we apply HLA-DQB1*03:01 as a risk predictor for noninflammatory DPP-4i-BP in the Japanese population. In addition to the allele frequencies, the two- or three-locus haplotype frequencies for HLA-DQA1, -DQB1 and -DRB1 were compared between DPP-4i-BP and control groups. HLA-DRB1*12:01-DQB1*03:01 showed the lowest *P*-value

Abbreviations: BP, bullous pemphigoid; BPDAI, Bullous Pemphigoid Disease Area Index; COL17, type XVII collagen; DPP-4i, dipeptidyl peptidase-4 inhibitor

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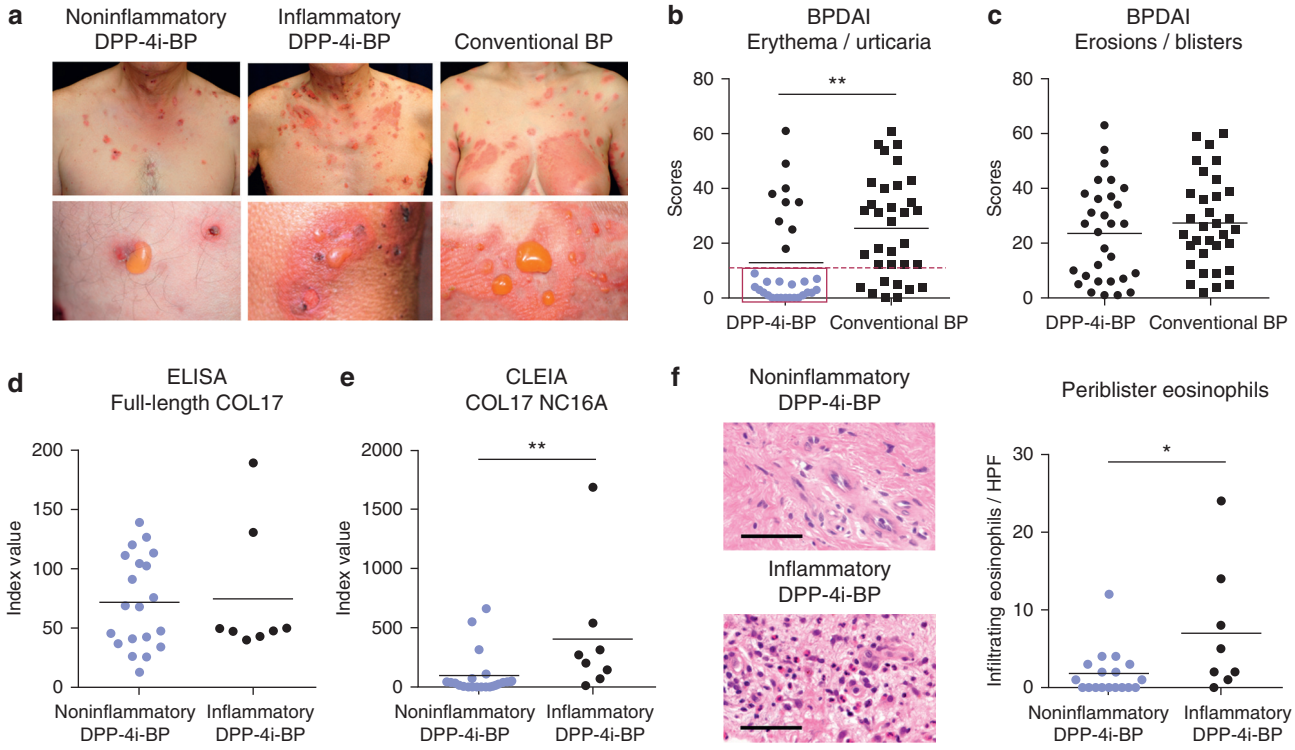

Figure 1. DPP-4i-BP shows a unique noninflammatory phenotype with few erythematous lesions. (**a**) The clinical appearance of noninflammatory DPP-4i-BP, inflammatory DPP-4i-BP, and conventional BP. (**b**) BPDAI scores for erythema/urticaria. The dashed line indicates a BPDAI (erythema/urticaria) score of 10. The red square indicates a group of patients with noninflammatory DPP-4i-BP. The blue dots represent scores of noninflammatory DPP-4i-BP. (**c**) BPDAI scores for erosions/blisters. (**d**) Full-length COL17 ELISA index. (**e**) COL17 NC16A CLEIA index. (**f**) Histopathological findings of representative noninflammatory DPP-4i-BP and inflammatory BP. Hematoxylin and eosin staining, scale bar = 100 μm. (**d**) Comparison of the number of infiltrating eosinophils between noninflammatory DPP-4i-BP and inflammatory BP. Bars represent mean values. $*P < 0.05$, $**P < 0.01$ using Mann-Whitney test. BP, bullous pemphigoid disease; BPDAI, Bullous Pemphigoid Disease Area Index; CLEIA, chemiluminescent enzyme immunoassay; COL17, type XVII collagen; DPP-4i, dipeptidyl peptidase-4 inhibitor; HPF, high-powered field.

in 243 haplotypes ($P = 2.16 \times 10^{-8}$), which was greater than that of HLA-DQB1*03:01 alone ($P = 5.86 \times 10^{-11}$), indicating that HLA-DQB1*03:01 will be the more useful biomarker in predicting DPP-4i-BP before administration to Japanese patients (Table 1).

Six patients with conventional BP suffered from type 2 diabetes at the onset of BP. We found that BPDAI scores for erosions/blisters were similar in those with DPP-4i-BP and conventional BP with diabetes, whereas scores for erythema/urticaria were significantly higher in those with conventional BP with diabetes (see Supplementary Figure S1 online), suggesting that the noninflammatory phenotype in DPP-4i-BP correlates with the intake of DPP-4i rather than with the existence of type 2 diabetes. Furthermore, none of the patients with conventional BP with diabetes carried HLA-DQB1*03:01.

Eight patients with conventional BP had noninflammatory disease, and 37.5% (3/8) of those patients carried HLA-DQB1*03:01. This frequency is similar to that for patients with inflammatory conventional BP (16/64 [25%]) and inflammatory DPP-4i-BP (4/9 [44%]) and lower than that for those with noninflammatory DPP-4i-BP (18/21 [86%]), suggesting that HLA-DQB1*03:01 is associated with noninflammatory DPP-4i-BP rather than with noninflammatory conventional BP or inflammatory DPP-4i-BP.

To our knowledge, the association of HLA-DQB1*03:01 with noninflammatory DPP-4i-BP is the strongest association that has been described between a class II HLA and a drug-related autoimmune disease. HLA-DQB1*03:01, also reported to be associated with mucous membrane pemphigoid in Caucasian patients (Ahmed et al., 1991; Delgado et al., 1996), seems to be a risk factor for DPP-4i-BP in Japanese. To confirm this, the incidence of DPP-4i-BP among diabetic patients carrying HLA-DQB1*03:01 should be investigated. In addition, to determine whether the noninflammatory phenotype is a distinctive feature of DPP-4i-BP or just a mild form of BP, further investigations are required. The findings of this study give us important clues about the breakdown of self-tolerance that results from the interaction of genetic background and drug intake.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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Table 1. Frequency of HLA alleles and haplotypes in cases and controls

HLA Allele	DPP-4i-BP (Noninflammatory), n (%) (n = 21)	Conventional BP, n (%) (n = 72)	Japanese General Population Control Individuals, n (%) (n = 873)	DPP-4i−Tolerant Diabetes Patients, n (%) (n = 61)	DPP-4i-BP (Noninflammatory) Patients Versus General Population Control Individuals		Conventional BP Patients Versus General Population Control Individuals		DPP-4i-BP (Noninflammatory) Versus Tolerant Control Individuals	
					OR (95% CI)	*P*-Value[1]	OR (95% CI)	*P*-Value	OR (95% CI)	*P*-Value[2]
DQB1*03:01	18 (86)	19 (26)	156 (18)	19 (31)	27.6 (8.0–94.8)	5.86×10^{-11}	1.6 (0.9–2.9)	8.24×10^{-2}	13.3 (3.5–50.5)	**2.13×10^{-5}**
DQA1*05:05	10 (48)	10 (14)	60 (7)	11 (18)	12.3 (5.0–30.2)	8.11×10^{-7}	2.2 (1.1–4.5)	5.58×10^{-2}	4.1 (1.4–12.1)	1.79×10^{-2}
DRB1*12:01	10 (48)	8 (11)	68 (8)	7 (11)	10.8 (4.4–26.2)	2.34×10^{-6}	1.5 (0.7–3.2)	3.63×10^{-1}	7.0 (2.2–22.4)	**1.08×10^{-3}**
DQA1*01:03	0 (0)	18 (25)	368 (42)	18 (30)	0.0 (0.0–0.5)	1.52×10^{-5}	0.5 (0.3–0.8)	4.07×10^{-3}	0.1 (0.0–0.9)	**4.31×10^{-3}**
DRB1*11:01	7 (33)	6 (8)	37 (4)	9 (15)	11.3 (4.3–29.7)	2.99×10^{-5}	2.1 (0.8–5.0)	1.32×10^{-1}	2.9 (0.9–9.1)	1.07×10^{-1}
DQB1*06:01	0 (0)	18 (25)	359 (41)	18 (30)	0.0 (0.0–0.6)	3.67×10^{-5}	0.4 (0.3–0.8)	3.45×10^{-2}	0.1 (0.0–0.9)	**4.31×10^{-3}**
DRB1*12:01-DQB1*03:01	10 (48)	6 (8)	39 (4)	1 (0)	19.4 (7.8–48.5)	2.16×10^{-8}	1.9 (0.8–4.8)	1.46×10^{-1}	54.5 (6.3–470.1)	**1.56×10^{-6}**
DQA1*05:05-DQB1*03:01	10 (48)	8 (11)	58 (7)	11 (18)	12.8 (5.2–31.3)	6.09×10^{-7}	1.8 (0.8–3.8)	1.51×10^{-1}	4.1 (1.4–12.1)	1.79×10^{-2}
DQA1*01:03-DQB1*06:01	0 (0)	16 (22)	357 (41)	18 (30)	0.0 (0.0–0.6)	3.65×10^{-5}	0.4 (0.2–0.7)	1.62×10^{-3}	0.0 (0.0–0.9)	**4.31×10^{-3}**

Abbreviations: BP, bullous pemphigoid; CI, confidence interval; DPP-4i, dipeptidyl peptidase-4 inhibitor; HLA, human leukocyte antigen; OR, odds ratio.
[1]All values in this column are significant after Bonferroni correction: $P < 1.27 \times 10^{-4}$ (0.05/152 HLA-A, -B, -C, -DRB1, -DPB1, -DQA, -DQB1 alleles; 76 DRB1-DQA1-DQB1 haplotypes; 52 DRB1-DQA1 haplotypes; 68 DRB1-DQB1 haplotypes; and 47 DQA1-DQB1 haplotypes).
[2]Boldface values in this column are significant after Bonferroni correction: $P < 5.56 \times 10^{-3}$ (0.05/6 HLA alleles, 1 DRB1-DQB1 haplotype, and 2 DQA1-DQB1 haplotypes).

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2017.11.023.

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Variability in the Expression of Immunohistochemical Markers: Implications for Biomarker Interpretation in Cutaneous T-Cell Lymphoma

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TO THE EDITOR

Clinical development of targeted therapies in cutaneous T-cell lymphoma compels us to explore biomarkers that may correlate with clinical outcome. In contrast to the uniform distribution of Sézary cells in the blood, the skin compartment in mycosis fungoides (MF) and Sézary syndrome (SS) is often heterogeneous, with malignant T cells variably admixed with the resident and infiltrating non-malignant cells across the varying morphologic types of skin lesions, including patches, plaques, and tumors (Olsen et al., 2011; Swerdlow et al., 2016). Thus, collecting a single skin biopsy in a patient with MF/SS may lead to misinterpretation of biomarker information. Recently, the phase 3 trial of brentuximab vedotin (antibody drug conjugate against CD30) versus physician's choice (methotrexate or bexarotene) in CD30-positive cutaneous T-cell lymphoma reported notable variability in CD30 expression among multiple biopsies collected at baseline for patients with MF (Kim et al., 2017). In that study, approximately one-third of patients with MF were excluded during screening using an arbitrary cutoff of 10% CD30 expression (Kim et al., 2017). Here, we conducted an in-depth evaluation of the variability of CD30 and other potential tissue biomarkers in the skin as assessed by immunohistochemistry (IHC), which was a planned exploratory objective in our phase 2 investigator-initiated trial of brentuximab vedotin in patients with MF/SS (Kim et al., 2015). The primary goal was to assess the variability of IHC marker expression levels in different skin lesions in the same patient (intra-patient, inter-lesional) and within a lesion (intra-lesional). The secondary goal was to evaluate the variability of IHC marker expression in different clinical types of lesions (patch/plaque vs. tumor) and in those biopsy samples with and without large cell transformation.

We evaluated CD30 and other potential tissue biomarker expression in 144 biopsy samples obtained from 36 patients at baseline (Supplementary Figure S1). The study was approved by the Institutional Review Board at Stanford University (NCT01396070; IRB21324). Written informed consent was obtained for each patient. Tissue IHC was performed using standard protocols (Supplementary Table S1). A board-certified dermatopathologist (J.K.) reviewed all final slides. We evaluated the variability in the biomarker expression using intraclass correlation coefficient (ICC) and delta. ICC reflects the degree of correlation and agreement between measurements, where values closer to 1.0 represent stronger agreement (Koo and Li, 2016). An ICC of <0.4 was considered poor, 0.4–0.7 was considered acceptable, while an ICC >0.7 was considered excellent correlation (Shrout and Fleiss, 1979). The inter-lesional ICC demonstrated the agreement in the expression of biomarkers between two samples from different lesions in the same patient and intra-lesional ICC demonstrated the agreement in the expression levels between two samples within the same lesion. Delta was defined as the difference between maximum and minimum expression of the biomarker among all biopsy samples in the same patient. Of the 36 patients enrolled in the study, the majority had the diagnosis of MF (31 MF, 5 SS) and 86% of patients had advanced disease (20 stage IIB, 11 stage IV). The median number of skin biopsies per patient was 4 (range 2–7). Eighty-one percent of the biopsies were from plaque- or tumor-type (81 plaque, 35 tumor) lesions. Large cell transformation was detected in 21% (30 of 144) of the biopsy samples. We observed varying histomorphology in the biopsy samples with the tumor-type lesions more likely to show diffuse pattern of mononuclear cell infiltrate (100%) and large cell transformation (43%). Figure 1a highlights the inter- and intra-patient variability in the expression of selected IHC markers among multiple biopsies for each patient. Nondetectable CD30 expression (0%) was reported in at least one biopsy sample from a patient in 17% (6 of 36) of patients. In these patients, the maximum CD30 expression ranged from 1% to 40% (median 5%) among 4–7 biopsy samples. Table 1 summarizes the estimates for intra-lesional ICC, inter-lesional ICC, and the median delta (range) across all patients for

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