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# Variants at *HLA-A*, *HLA-C*, and *HLA-DQB1* Confer Risk of Psoriasis Vulgaris in Japanese



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Psoriasis vulgaris (PsV) is an autoimmune disease of skin and joints with heterogeneity in epidemiologic and genetic landscapes of global populations. We conducted an initial genome-wide association study and a replication study of PsV in the Japanese population (606 PsV cases and 2,052 controls). We identified significant associations of the single nucleotide polymorphisms with PsV risk at *TNFAIP3-interacting protein 1* and the major histocompatibility complex region ( $P = 3.7 \times 10^{-10}$  and  $6.6 \times 10^{-15}$ , respectively). By updating the HLA imputation reference panel of Japanese ( $n = 908$ ) to expand HLA gene coverage, we fine-mapped the HLA variants associated with PsV risk. Although we confirmed the PsV risk of HLA-C\*06:02 (odds ratio = 6.36,  $P = 0.0015$ ), its impact was relatively small compared with those in other populations due to rare allele frequency in Japanese (0.4% in controls). Alternatively, HLA-A\*02:07, which corresponds to the cysteine residue at HLA-A amino acid position 99 (HLA-A Cys99), demonstrated the most significant association with PsV (odds ratio = 4.61,  $P = 1.2 \times 10^{-10}$ ). In addition to HLA-A\*02:07 and HLA-C\*06:02, stepwise conditional analysis identified an independent PsV risk of HLA-DQB1 Asp57 (odds ratio = 2.19,  $P = 1.9 \times 10^{-6}$ ). Our PsV genome-wide association study in Japanese highlighted the genetic architecture of PsV, including the identification of HLA risk variants.

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## INTRODUCTION

Psoriasis vulgaris (PsV) is a chronic immune-mediated disease of skin and joints characterized by inflammation, epidermal hyperplasia, and vascular remodeling (Nestle et al., 2009). PsV is a heritable trait, and both

environmental and genetic factors contribute substantially to the development of PsV (Nestle et al., 2009). To date, large-scale genome-wide association studies (GWAS) have identified more than 60 genetic loci associated with PsV risk (e.g., *IL23R*, *REL*, *IL12B*, *TNFAIP3*, and *ERAP1*). The functional annotation of these loci implicated biological pathways related to disease biology of PsV including IL signaling and an NF- $\kappa$ B pathway (Tang et al., 2014; Tsoi et al., 2015, 2017; Sheng et al., 2014; Yin et al., 2015; Zuo et al., 2015). In addition to the common single nucleotide polymorphisms (SNPs) identified by GWAS, rare mutations associated with familial forms of PsV were detected by sequence analyses (Hayashi et al., 2014; Jordan et al., 2012).

As observed in other immune-related diseases, the major histocompatibility complex (MHC) region at 6p21 confers the strongest genetic risk of PsV. PsV risk within MHC was originally mapped to a critical region at MHC class I, termed *PSORS1* (Tiilikainen et al., 1980); then, HLA-C\*06:02 was identified as the causal allele that explains the risk of *PSORS1* (Nair et al., 2006). The strong PsV risk of HLA-C\*06:02 allele has been validated in worldwide populations including Europeans (Lenz et al., 2015; Okada et al., 2014a), South Asians (Chandra et al., 2016), and East Asians (Mabuchi et al., 2014; Zhou et al., 2016), with an odds ratio (OR) of as high as 3.0–10.0. However, the genetic landscape of PsV within MHC has yet to be elucidated. HLA fine-mapping analysis using the HLA imputation method successfully identified multiple HLA variants with PsV risk independently from HLA-C\*06:02 (Okada et al., 2014a). For example, HLA imputation analysis in Europeans reported that amino acid polymorphisms in *HLA-A*, *HLA-B*, and *HLA-DQA1* confer independent PsV risk. Thus,

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Abbreviations: CI, confidence interval; GWAS, genome-wide association study; MAF, minor allele frequency; MHC, major histocompatibility complex; OR, odds ratio; PsV, psoriasis vulgaris; QC, quality control; SNP, single nucleotide polymorphism

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further HLA fine-mapping studies of PsV in other populations are warranted.

Epidemiologic studies reported that the prevalence and incidence of PsV shows ethnic and geographic variations (Chandran et al., 2010; Parisi et al., 2013). Although a relatively high prevalence is observed in European countries and in the USA (0.5–6.5%), the prevalence in East Asian countries is low (0.2–0.3%) (Chandran et al., 2010; Parisi et al., 2013). Because of the strong PsV risk of HLA-C\*06:02, global heterogeneity of PsV is considered to be partially explained by the allele frequency spectra of HLA-C\*06:02. There appears to be a lower HLA-C\*06:02 prevalence, and consequently a lower rate of PsV, within the populations whose ancestors migrated to the orient (Chandran et al., 2010, 2016). From this perspective, the Japanese population has unique characteristics. PsV prevalence in Japan is one of the lowest compared with worldwide populations (0.1–0.3%) (Kubota et al., 2015; Naito et al., 2016; Takahashi et al., 2009), and the frequency of HLA-C\*06:02 is almost rare within the Japanese population (<0.5%) (Mabuchi et al., 2014). This may suggest that the Japanese patients with PsV have a higher proportion of HLA-C\*06:02-negative individuals; thus, genetic studies investigating the Japanese population could contribute to unveiling novel genetic architecture associated with PsV. However, a genome-wide assessment of PsV risk has not been conducted in the Japanese population to date.

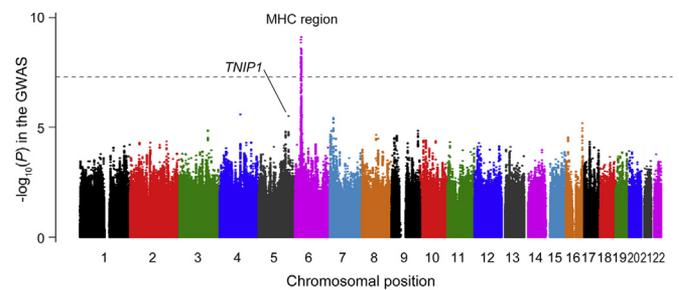
In this study, we conducted an initial GWAS of PsV and a replication study within the Japanese population (in total 606 PsV cases and 2,052 controls). We then applied the HLA imputation method to the GWAS data by updating the HLA reference panel of the Japanese population to comprehensively fine-map the risk of the HLA variants (n = 908).

## RESULTS

### PsV GWAS in the Japanese population

In the PsV GWAS, 282 PsV cases and 426 controls were enrolled from the Japanese population. Genotyping of the genome-wide SNPs was performed using Illumina HumanCoreExome BeadChip (Illumina, San Diego, CA). We applied stringent quality control (QC) filters, and obtained 255,632 autosomal SNPs. To extend the coverage of the SNPs, we conducted whole-genome SNP genotype imputation using a multiethnic reference panel of the 1000 Genomes Project (phase 3v5; n = 2,504). After SNP imputation, 6,369,185 autosomal SNPs that satisfied the criteria of minor allele frequency (MAF)  $\geq 0.5\%$  and imputation score ( $R_{sq}$  by minimac2)  $\geq 0.7$  were obtained. Associations of the SNPs with PsV risk were evaluated using a logistic regression model.

Quantile-quantile plots of the genome-wide  $P$ -values indicated no remarkable inflation of test statistics both before and after SNP imputation ( $\lambda_{GC} = 1.007$  and 1.003, respectively; Supplementary Figure S1 online), which suggested that no substantial population stratification exists in the studied subjects. As observed in the previous PsV GWAS in other populations (Sheng et al., 2014; Tang et al., 2014; Tsoi et al., 2015, 2017; Yin et al., 2015; Zuo et al., 2015), the most significant association signal that satisfied the genome-wide significance threshold of  $P < 5.0 \times 10^{-8}$  (Kanai et al., 2016) was observed within the MHC region



**Figure 1. A Manhattan plot of the genome-wide association study (GWAS) of psoriasis vulgaris in the Japanese population.** A Manhattan plot showing  $-\log_{10}(P)$  of the genome-wide single nucleotide polymorphisms (SNPs) in the GWAS of psoriasis vulgaris in the Japanese population (after SNP imputation). The black horizontal line represents the genome-wide significance threshold of  $P = 5.0 \times 10^{-8}$ . The genetic loci that satisfied the genome-wide significance threshold in the combined analysis of the GWAS and the replication study are labeled. MHC, major histocompatibility complex.

( $P = 1.0 \times 10^{-8}$  at rs3132506 and  $P = 2.8 \times 10^{-9}$  at rs378352 before and after SNP imputation, respectively; Figure 1 and Supplementary Figure S2 online).

### Replication study

To further explore the PsV risk SNPs in the Japanese population, we conducted a replication study that enrolled an independent PsV case-control cohort of Japanese individuals (320 PsV cases and 1,622 controls). We selected 22 loci that satisfied  $P < 5.0 \times 10^{-4}$  in the GWAS, and performed genotyping of the lead SNP of each loci (Supplementary Table S1 online). Of these, SNPs in two loci (rs2233278 at TNFAIP3-interacting protein 1 and rs9394026 at the MHC region) showed nominal association signals with the same directional effects that were observed in the GWAS ( $P = 7.0 \times 10^{-6}$  and  $9.6 \times 10^{-8}$ , respectively). *TNFAIP3-interacting protein 1* is a known PsV risk locus implicated in regulation of an NF- $\kappa$ B pathway and microRNA (Okada et al., 2016a; Olarerin-George et al., 2013), and an association of rs2233278 with PsV was reported in the Japanese population (Tamari et al., 2014). After the meta-analysis combining the GWAS and the replication study, these two SNPs demonstrated associations that satisfied the genome-wide significance threshold (OR = 1.96, 95% confidence interval [95% CI] = 1.59–2.41,  $P = 3.7 \times 10^{-10}$  for rs2233278 at TNFAIP3-interacting protein 1, and OR = 1.86, 95% CI = 1.59–2.18,  $P = 6.6 \times 10^{-15}$  for rs378352 at the MHC region; Table 1).

### Update of the HLA imputation reference panel of Japanese

To fine-map the risk of HLA variants of PsV in the Japanese population, we applied the HLA imputation method to the PsV GWAS data using the previously constructed Japanese population-specific reference panel (n = 908) (Okada et al., 2015, 2016b; Shiraishi et al., 2016). To expand the coverage of the HLA genes for imputation, we newly genotyped the *HLA-DQA1* alleles of the subjects in the reference panel. The updated reference panel included three class I HLA genes (*HLA-A*, *HLA-B*, and *HLA-C*) and five class II HLA genes (*HLA-DRB1*, *HLA-DQA1*, *HLA-DQB1*, *HLA-DPA1*, and *HLA-DPB1*). Empirical evaluation of imputation accuracy based on a cross-validation approach demonstrated high

**Table 1. SNPs with genome-wide significant associations with psoriasis vulgaris risk in Japanese**

rsID	Chr	Position (hg19)	Allele 1/2	Gene	Study stage	No. of subjects		Allele 1 freq.		PsV association	
						PsV	Control	PsV	Control	OR (95% CI)	P
rs2233278	5	150,467,189	C/G	TNIP1	GWAS	282	426	0.138	0.070	2.62 (1.75–3.92)	$3.0 \times 10^{-6}$
					Replication	313	1,620	0.157	0.095	1.76 (1.37–2.25)	$7.0 \times 10^{-6}$
					Meta-analysis	595	2,046	0.148	0.090	1.96 (1.59–2.41)	$3.7 \times 10^{-10}$
rs9394026	6	30,982,544	G/A	MHC region	GWAS	282	426	0.303	0.168	2.25 (1.72–2.95)	$2.8 \times 10^{-9}$
					Replication	313	1,620	0.302	0.204	1.69 (1.39–2.04)	$9.6 \times 10^{-8}$
					Meta-analysis	595	2,046	0.302	0.197	1.86 (1.59–2.18)	$6.6 \times 10^{-15}$

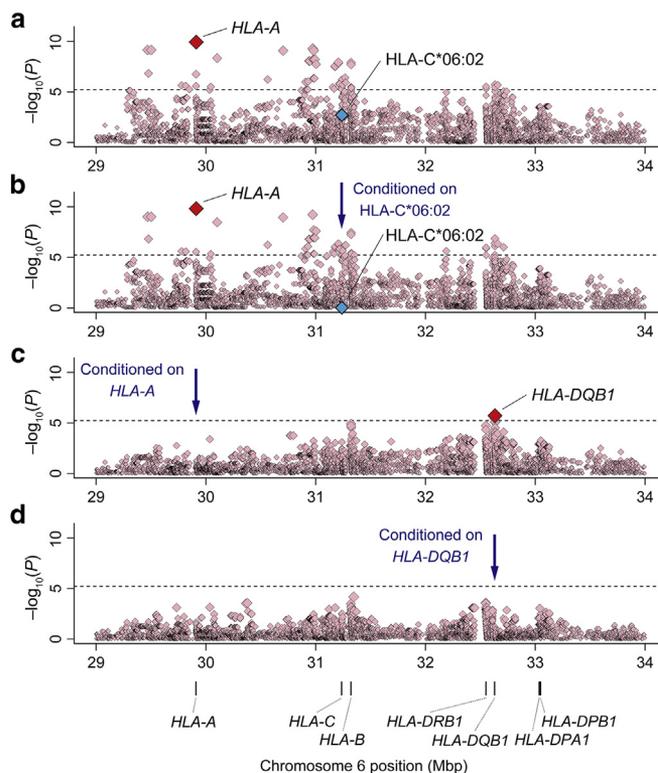
Abbreviations: CI, confidence interval; GWAS, genome-wide association study; MHC, major histocompatibility complex; OR, odds ratio; PsV, psoriasis vulgaris; SNP, single nucleotide polymorphism.

concordance rates between imputed and genotyped HLA alleles (on average 98.4% and 95.9% for the two-digit and four-digit alleles, respectively; Supplementary Table S2 online).

**HLA imputation of the PsV GWAS data**

We imputed HLA variants of the PsV GWAS data using the updated reference panel of Japanese and SNP2HLA (Jia et al., 2013; Okada et al., 2014b; Raychaudhuri et al., 2012), one of the most accurate HLA imputation programs (Karnes et al., 2017). After the application of postimputation QC filters (MAF  $\geq 0.1\%$  and  $\geq 0.5\%$  for the HLA variants and the SNPs, respectively, and an imputation score  $R_{sq} \geq 0.5$ ), we obtained genotypes of 71 two-digit HLA alleles, 117 four-digit HLA alleles, 958 amino acid residues, and 6,477 SNPs in the MHC region (from 29 to 34 Mbp at chromosome 6, NCBI build 37). We assessed the PsV risk of each imputed variant with a binary test ( $n = 7,623$ ) and each amino acid position of the HLA genes by an omnibus test ( $n = 688$ ). We set the regional significance threshold according to Bonferroni's correction based on the number of tests ( $\alpha = 0.05$ ;  $P = 0.05/8,311 = 6.0 \times 10^{-6}$ ).

Interestingly, the most significant PsV association within MHC was observed not at HLA-C\*06:02, but at HLA-A\*02:07, which corresponds to a cysteine residue of HLA-A amino acid position 99 in Japanese (OR = 4.61, 95% CI = 2.79–7.61,  $P = 1.2 \times 10^{-10}$ ; Figure 2a) that satisfied the region-wide significance threshold. Although we observed an association of HLA-C\*06:02 (OR = 6.36, 95% CI = 1.70–23.8,  $P = 0.0015$ ), its impact on PsV susceptibility was less apparent compared with those observed in other populations due to low allele frequency in the Japanese population (2.3% in PsV cases and 0.4% in controls). We note that HLA-C\*12:03, another HLA-C risk allele of PsV in Europeans, was also rare in Japanese (0.1% in controls). Although HLA-C\*12:03 showed the same directional effect as observed in Europeans, its association was not significant in Japanese (OR = 7.41, 95% CI = 0.47–117.6,  $P = 0.13$ ). When conditioned on HLA-C\*06:02, HLA-A\*02:07 (and HLA-A Cys99) still demonstrated significant PsV risk (OR = 4.61, 95% CI = 2.79–7.63,  $P = 1.5 \times 10^{-10}$ ; Figure 2b). No other HLA-A variant showed a significant association when conditioned on HLA-C\*06:02 and HLA-A\*02:07 ( $P > 0.077$ ), which suggested that the PsV risk of HLA-A in Japanese could be explained by that of HLA-A\*02:07 (and HLA-A Cys99).



**Figure 2. Regional associations of the variants in the major histocompatibility complex (MHC) region with psoriasis vulgaris risk.**

Regional associations of the variants in the MHC region with psoriasis vulgaris risk in the Japanese population estimated based on the HLA imputation analysis. (a) Nominal regional associations. (b–d) Stepwise analysis results conditioned on HLA-C\*06:02, HLA-A, and HLA-DQB1. Each diamond represents the  $-\log_{10}(P)$  of the variants, including the single nucleotide polymorphism, two-digit and four-digit HLA alleles, and amino acid polymorphisms of HLA genes. The dotted horizontal line represents the region-wide significance threshold of  $P = 6.0 \times 10^{-6}$ . The most strongly associated variant in each panel is labeled.

Alternatively, when we conditioned on HLA-A first, we also observed an association of HLA-C\*06:02 ( $P = 0.0018$ ).

To further explore additional HLA variants that confer independent PsV risk, we conducted a conditional analysis on HLA-C\*06:02 and HLA-A. We observed a significant association in the MHC class II region, which was highlighted at an aspartic acid residue of HLA-DQ $\beta$ 1 amino acid position

**Table 2. Associations of the HLA variants for psoriasis vulgaris risk in Japanese**

Variant	Frequency		OR (95% CI)	P	Explained risk in the population
	PsV	Control			
HLA-A*02:07 <sup>1</sup>	0.112	0.028	4.19 (2.52–6.97)	$3.4 \times 10^{-8}$	0.013
HLA-C*06:02	0.023	0.0040	10.27 (2.59–40.73)	$9.2 \times 10^{-4}$	0.0054
HLA-DQβ1 57Asp	0.825	0.729	1.98 (1.47–2.67)	$7.4 \times 10^{-6}$	0.018

Abbreviations: CI, confidence interval; OR, odds ratio; PsV, psoriasis vulgaris.

<sup>1</sup>Equivalent to HLA-A Cys99 in the Japanese population.

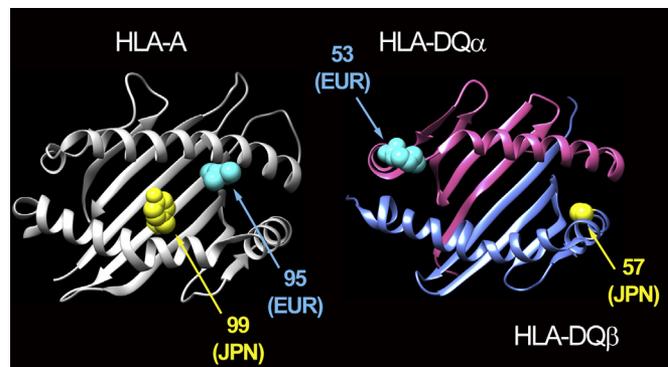
57 (OR = 2.19, 95% CI = 1.57–3.07,  $P = 1.9 \times 10^{-6}$ ; Figure 2c). No other variants in the MHC region satisfied the region-wide significance threshold when conditioned on HLA-C\*06:02, HLA-A, and HLA-DQβ1 Asp57 (or HLA-DQB1; Figure 2d), whereas suggestive associations of other HLA genes such as HLA-B (the smallest  $P = 5.8 \times 10^{-4}$  and  $P = 7.1 \times 10^{-4}$  at HLA-B\*15:18 and HLA-B Cys67, respectively) or HLA-DRB1 (the smallest  $P = 2.5 \times 10^{-4}$  at HLA-DRβ1 amino acid position 149) were observed. Of these, HLA-B Cys67 was reported as a PsV risk variant in Europeans (Okada et al., 2014a).

In summary, imputation-based HLA fine-mapping analysis demonstrated that the PsV risk of MHC could be explained by combinations of the multiple class I and class II HLA genes in the Japanese population (HLA-C\*06:02, HLA-A\*02:07 [i.e., HLA-A Cys99], and HLA-DQβ1 Asp57). A multivariate full regression model including these risk variants (Table 2) explained 3.6% of the overall PsV risk in the population under the assumption of 0.2% of disease prevalence (Kubota et al., 2015; Naito et al., 2016; Takahashi et al., 2009). As expected, the impact of HLA-C\*06:02 (0.54%) was relatively smaller than those of HLA-A\*02:07 and HLA-DQβ1 Asp57 (1.3% and 1.8%, respectively). We did not observe non-additive effects of the risk variants ( $P_{\text{nonadditive}} > 0.43$ ). Although localization of HLA-C\*06:02 to specific amino acid positions of HLA-C is known to be difficult (Clop et al., 2013; van den Bogaard et al., 2016; Vince et al., 2016), PsV risk amino acid polymorphisms of HLA-A Cys99 and HLA-DQβ1 Asp57 were located at functional pockets of HLA-A and HLA-DQ molecules (Figure 3), suggesting their biological impacts.

## DISCUSSION

In this study, we conducted an initial GWAS of PsV in the Japanese population. Combined analysis of the GWAS and the replication study confirmed PsV risk at *TNFAIP3-interacting protein 1* and the MHC region. We further performed HLA risk fine-mapping analysis by updating the HLA imputation reference panel, and found that combinations of the multiple HLA variants at class I and class II HLA genes (HLA-C\*06:02, HLA-A\*02:07 [i.e., HLA-A Cys99], and HLA-DQβ1 Asp57) explained PsV risk within the MHC region in Japanese.

Although HLA-C\*06:02 has shown the strongest association with PsV in other populations (Lenz et al., 2015; Okada et al., 2014a; Zhou et al., 2016), its impact in the Japanese population was relatively small due to rare allele frequency (<0.5%). Our study has identified for the first time a population in which HLA-C\*06:02 is not the strongest genetic risk



**Figure 3. Psoriasis vulgaris risk amino acid positions of HLA genes in three-dimensional ribbon models.** HLA amino acid positions with psoriasis vulgaris risk in HLA-A and HLA-DQ molecules are indicated in three-dimensional ribbon models. The protein structures of HLA-A and HLA-DQ are based on Protein Data Bank entries 1x7q and 1jk8, respectively, and prepared using UCSF Chimera (version 1.7). Residues at amino acid positions with PsV risk in Japanese (this study) and Europeans (Okada et al., 2014a) are highlighted in yellow and blue, respectively.

factor for psoriasis. We note that our large-scale imputation reference panel achieved high imputation quality of HLA-C\*06:02 despite its low frequency ( $Rsq = 0.91$ ). A leave-one-out cross-validation empirically demonstrated 100% of sensitivity in imputation of HLA-C\*06:02. These results might reject the possibility that the impact of HLA-C\*06:02 was underestimated due to inaccurate imputation. Previous studies reported that the PsV risk of the HLA-C variants such as HLA-C\*06:02 and HLA-C\*12:02 is associated with age of onset (Bowes et al., 2017; Mabuchi et al., 2014). Further stratified analysis based on age of onset would be warranted to comprehensively elucidate genetic landscape of PsV in the Japanese population.

Alternatively, HLA-A\*02:07 demonstrated the most significant association with PsV in Japanese. Associations of HLA-A\*02 with PsV and psoriatic arthritis were previously reported in Japanese (Muto et al., 1995; Nakagawa et al., 1991), which was subsequently found to be driven by an association of HLA-A\*02:07 (Muto et al., 1996). Previous studies in the Chinese population suggested the PsV risk of HLA-A\*02:07, but this result was met with controversial discussions (Yin et al., 2015; Zhou et al., 2016). Our study robustly confirmed the PsV risk of HLA-A\*02:07 that satisfied the genome-wide significance threshold. Although HLA-A\*02:07 has a variety of allele frequency spectra in world-wide populations, its frequency in Japanese (=0.028) is

relatively higher than those in other populations (e.g., 0.020 in East Asians and 0.0028 in Europeans) (Okada et al., 2015). HLA-A\*02:07 corresponds to HLA-A Cys99 in the Japanese population. A previous MHC fine-mapping study in Europeans reported the PsV risk of HLA-A amino acid position 95, which is closely located to the risk amino acid position 99 identified in the Japanese population (Figure 3). This may suggest that HLA-A amino acid positions around 95–99 have biological impacts on PsV pathogenicity.

We further identified the PsV risk amino acid polymorphism of HLA-DQβ1 (Asp57). A previous study in Europeans pointed PsV risk amino acid polymorphism of HLA-DQα1, proposing the hypothesis that HLA-DQ molecules might also have crucial roles in PsV disease biology (Okada et al., 2014a). The PsV risk of HLA alleles that carry HLA-DQβ1 Asp57 has been reported in previous studies in Japanese (Nakagawa et al., 1990; Saeki et al., 1998). We also note that HLA-DQβ1 Asp57 is known to be associated with type 1 diabetes mellitus in European (Hu et al., 2015). These results should implicate that the amino acid position 57 has critical functional roles in diseases biology.

In conclusion, our initial PsV GWAS in the Japanese population highlighted genetic architecture of PsV including identification of novel HLA risk variants, which was different from previous findings in other populations. Our study should motivate further accumulation of genetic studies from a variety of populations to further elucidate the disease biology of PsV.

## MATERIAL AND METHODS

### Subjects

We enrolled 606 PsV cases and 2,052 controls for the GWAS and the replication study. Genomic DNA from the PsV cases and the controls enrolled in the GWAS ( $n = 282$  [mean  $\pm$  standard deviation age =  $51.7 \pm 15.2$ , female ratio = 0.20] and 426 [mean  $\pm$  standard deviation age =  $38.2 \pm 0.4$ , female ratio = 0.45], respectively) were obtained from Epstein-Barr virus transformed B-lymphoblast cell lines of unrelated Japanese individuals established by the Japan Biological Informatics Consortium. The PsV cases in the replication study ( $n = 324$  [mean  $\pm$  standard deviation age =  $53.8 \pm 15.3$ , female ratio = 0.26]) were collected from three medical institutes in Japan (the Jikei University School of Medicine, Nippon Medical School, and the University of Tokyo) (Tamari et al., 2014). The controls in the replication study ( $n = 1,626$  [mean  $\pm$  standard deviation age =  $51.7 \pm 11.1$ , female ratio = 0.41]) comprised DNA samples from healthy volunteers collected at the Midosuji and other related rotary clubs of Japan ( $n = 622$ ) and Tsukuba University ( $n = 1,004$ ). PsV was diagnosed in patients via clinical and histopathological findings. The genomic DNA was prepared in accordance with standard protocols. We enrolled 908 healthy controls used in our previous study for the construction of the HLA imputation reference panel (Okada et al., 2015). A part of these 908 subjects were also included in the controls of the replication study, but not included in the GWAS subjects. All subjects were of Japanese origin and provided written informed consent that was approved by the ethical committee of each institute.

### Statistical power calculation

We estimated statistical power of our study to achieve the genome-wide significance threshold ( $\alpha = 5.0 \times 10^{-8}$ ), using a

*power.prop.test* function implemented in *R* statistical software (version 3.1.2). Numbers of the subjects used for power estimates were harmonic means of the cases and controls in the GWAS or the meta-analysis of the GWAS and the replication study. The GWAS and the meta-analysis had power of 6.1% and 80.5% for the MAF in the controls = 0.20 and OR of the risk variant = 2.5, respectively (Supplementary Figure S3 online).

### Genotyping and quality control in the GWAS

In the GWAS, the PsV cases and controls were genotyped using illumina HumanCoreExome BeadChip (v1.1; Illumina). We applied stringent QC filters as described elsewhere (Okada et al., 2014c). First, we applied QC filters to the subjects: (i) exclusion of samples with low call rates ( $<0.99$ ), (ii) exclusion of outliers from the clusters of East Asian populations in principal component analysis that was conducted together with HapMap Phase II populations, (iii) exclusion of closely related subjects (identity by descent  $>0.15$ ). Second, we applied QC filters to the SNPs: exclusion of SNPs with low call rates ( $<0.99$ ), with low MAF ( $<0.5\%$  in cases or controls), with Hardy Weinberg equilibrium  $P$ -value  $< 1.0 \times 10^{-7}$ , or with more than 0.1 of frequency differences when compared with those in the 1000 Genomes Project (1KG) phase 3v5a Japanese (JPT) subjects. We also excluded indels or SNPs with duplicated positions. After applying the QC filters, we estimated principal components using linkage disequilibrium pruned whole-genome SNP genotype data, which were then used as covariates in the association analysis.

### Genotyping imputation and association analysis in the GWAS

We conducted whole-genome genotype imputation of the genotyped GWAS SNPs. We used a multiethnic reference panel of the 1KG phase 3v5a subjects ( $n = 2,504$ ). Prephasing of the GWAS data was performed using SHAPEIT2, and imputation was conducted using minimac2. We applied postimputation QC filters of MAF  $\geq 0.5\%$  in cases and controls, and an imputation score of  $R_{sq} \geq 0.7$ . Associations of the SNPs with PsV were assessed using logistic regression analysis with mach2dat. The top two principal components were included as covariates.

### Replication study

To gain statistical power to finally satisfy the genome-wide significance threshold in the meta-analysis of the GWAS and the replication study, we adopted  $P < 5.0 \times 10^{-4}$  as a threshold for SNP selection in the replication study. From the loci that satisfied  $P < 5.0 \times 10^{-4}$  in the GWAS, we selected lead SNPs for the replication study. SNP genotyping was conducted using the multiplex PCR-based Invader assay (Hologic, Marlborough, MA). Association analysis in the replication study was assessed using logistic regression implemented in *R* statistical software (version 3.1.2). Meta-analyses of the GWAS and the replication study were conducted using a fixed-effect inverse-variance method.

### HLA imputation of the GWAS data

Regarding the HLA imputation method, we adopted the HLA reference panel of the Japanese population that was used in previous studies ( $n = 908$ ) (Okada et al., 2015, 2016b; Shiraishi et al., 2016). In addition to the HLA genes originally incorporated in the reference panel (*HLA-A*, *HLA-B*, and *HLA-C* for class I; *HLA-DRB1*, *HLA-DQB1*, *HLA-DPA1*, and *HLA-DPB1* for class II), we newly genotyped two-digit and four-digit alleles of *HLA-DQA1* and integrated them into the panel to expand coverage of the HLA variants for imputation. Genotyping of the *HLA-DQA1* was performed by a

sequence-based method reported previously (Voorter et al., 2006) with minor modifications. Briefly, exon 1 and exons 2–5 of the *HLA-DQA1* were amplified separately, and then directly sequenced. Haplotype phasing was performed using Beagle (version 3.3.2), and deduced haplotypes were used to assign *HLA-DQA1* alleles by comparing with *HLA-DQA1* allelic sequences in the Immuno Polymorphism Database (Robinson et al., 2016). Imputation accuracy of the updated reference panel was empirically evaluated by a cross-validation approach. We randomly split the panel into two datasets. HLA variants from one of the datasets were masked, and then imputed using another dataset as a reference. Concordances between imputed and genotyped HLA variants were calculated. A leave-one-out cross-validation of HLA-C\*06:02 was conducted in the following steps. (i) For each of the subjects in the reference panel who had HLA-C\*06:02, we removed the subject from the reference panel. (ii) For each, we reconstructed the reference panel using the rest of the subjects and imputed the HLA alleles of the removed subject. (iii) We checked whether HLA-C\*06:02 was correctly imputed.

On the basis of the updated reference panel, we applied HLA imputation using SNP2HLA (Jia et al., 2013; Okada et al., 2014b; Raychaudhuri et al., 2012). We extracted the SNPs located in the MHC region (defined as from 29 to 34 Mbp at chromosome 6, NCBI build 37) from the GWAS data, and imputed genotypes of the alleles and amino acid polymorphisms of the HLA genes, as well as of the additional SNPs in the reference panel. We applied a postimputation QC filter of MAF  $\geq$  0.1% for the HLA variants and MAF  $\geq$  0.5% for the SNPs with an imputation score of  $R_{sq} \geq$  0.5.

### Fine-mapping analysis of the HLA variants

Associations of the HLA variants with PsV risk were evaluated using logistic regression implemented in *R* statistical software (version 3.1.2). We assumed additive effects of the allele dosages on the log-odds scale. We defined the HLA variants as biallelic SNPs in the MHC region, two-digit and four-digit biallelic HLA alleles, biallelic HLA amino acid polymorphisms corresponding to their respective residues, and multiallelic HLA amino acid polymorphisms for each amino acid position. To robustly account for potential population stratification, we included the top five principal components as covariates.

An omnibus *P*-value for each HLA amino acid site was calculated by a log-likelihood ratio test comparing the likelihood of the null model against the fitted model. The significance of the improvement in model fitting was evaluated by calculating the deviance, which follows a  $\chi^2$  distribution with  $m - 1$  degree(s) of freedom for an amino acid position with  $m$  residues. A significance threshold was set according to Bonferroni's correction based on the number of the tested variants within the MHC region ( $\alpha = 0.05$ ).

Conditional association analysis of the HLA variant(s) was conducted by additionally including the HLA variant genotypes as covariates. When conditioning on the HLA genes, we included all the two-digit and four-digit alleles as covariates. We applied a forward stepwise conditional analysis based on the regional significance threshold. Using the HLA variants selected from the conditional analysis results, we performed a multivariate regression analysis. A proportion of the overall PsV risk in the population (i.e., phenotypic variance) explained by the risk of HLA variants was estimated based on a liability threshold model under the assumption of a disease prevalence of 0.2% (Kubota et al., 2015; Naito et al., 2016; Takahashi et al., 2009). Nonadditive effects of the HLA

variants were evaluated by additionally including nonadditive genotype dosages of the HLA variants as covariates.

### Data availability

The PsV case-control GWAS data are deposited at National BioScience Database Center with the accession ID: hum0114.v1 (<https://humandbs.biosciencedbc.jp/hum0114-v1>). The updated HLA imputation reference panel is deposited at National BioScience Database Center with the accession ID: hum0028.v1 (<https://humandbs.biosciencedbc.jp/hum0028-v1>).

### CONFLICT OF INTEREST

The authors state no conflict of interest.

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### SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at [www.jidonline.org](http://www.jidonline.org), and at <https://doi.org/10.1016/j.jid.2017.10.001>.

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