HLA-DQβ1 amino acid position 87 and DQB1*0301 are associated with Chinese Han SLE

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1 | BACKGROUND

The major histocompatibility complex (MHC) region (chr 6:29–34 Mb) harbors the human leukocyte antigen (HLA) genes of which many are associated with autoimmune diseases (Fernando et al., 2008). The risk of autoimmunity conferred by HLA polymorphisms is likely the result of variation in amino acid residues at specific positions, which may alter the structure and function of presented peptides (Astill, Ellis, Arif, Tree, & Peakman, 2003; Lee, Wucherpfennig, & Wiley, 2001; van Lummel et al., 2014; Scally et al., 2013). For certain disease, specific amino acid positions within HLA molecules may play an important functional role. SLE is a heterogeneous disease characterized by autoantibody production and damage to multiple organs due to immune complexes and inflammation (Chung et al., 2011). A genetic contribution of the human leukocyte antigen (HLA) region to SLE has been supported by epidemiological studies and several genetic studies (Deng & Tsao, 2010; Lee et al., 2014). Genes in class II...
HLA regions are dominantly represented as SLE susceptibility loci especially in T cell dependent antibody responses. Professors have shown that HLA-DRB1 was significantly associated with autoantibody subsets in SLE patients (Connolly & Hakonarson, 2012). Then studies fine-mapped the primary association within the MHC locus with SLE to HLA-DR, and further narrowed it down to specific amino-acid positions. For example, the role of amino acid position 11-13-26 in HLA-DRβ1 for Korean SLE susceptibility has been established (Kim et al., 2014). However, the effects of the genetic architecture of the MHC region on SLE risk have not yet to be fully elucidated due to the complexity of the region, extended regions of linkage disequilibrium (LD), and a lack of statistical power. Al-Motwee et al. found that HLA-DQB1*06 was associated with Saudis SLE patients (Al-Motwee et al., 2013). SNP rs2187668 at HLA-DR3 was significantly associated with anti-dsDNA and was stronger of association in anti-dsDNA positive SLE subjects compared with negative ones (Chung et al., 2011). However, those loci don’t fully explain the HLA mediated risk of SLE, and did not investigate the functional amino acids due to lack of a reference panel suitable for imputing their genetic variants. Data from GWAS also have had insufficient variant density to define the association signals within the MHC. In this study, we used recently established Han-MHC reference panel (Zhou et al., 2016) imputing Chinese SLE GWAS data to identify potential independent amino acid positions.

2 | METHODS

2.1 | Ethical compliance

The study was approved by the relevant local Institutional Ethical Committees and informed consent was obtained from patients and families.

2.2 | SLE GWAS data

We extracted genome-wide SNPs of 1047 cases and 1205 controls subjects (Chinese mainland SLE, data set #1) and SNP data of 612 cases and 2193 controls individuals (Hong Kong SLE, data set #2) in previously SLE GWAS, and filtered using standard quality control criteria (Han et al., 2009; Yang et al., 2010) (Table S1), including SNP and sample call rate, exclusion of closely related relative and outliers in terms of ancestry, and SNP minor allele frequency (MAF) and Hardy–Weinberg equilibrium cutoffs.

2.3 | HLA imputation

We imputed classical HLA alleles, HLA amino acid residues and untyped SNPs from each data set by SNP2HLA and the new Han-MHC reference panel. Imputed markers with minor allele frequency (≥1%) and imputation quality (PLINK R2) ≥0.3 were used in disease association tests. Basing on the known amino acid sequences of classical alleles of HLA-A, -B, -C, -DPA1, -DPB1, -DQA1, -DQB1 and -DRB1 in the IMGT/HLA database (database release 3.13.1) (Kallberg et al., 2007; Robinson et al., 2015) translated the amino acid residues of each HLA genes. All information about the SNPs, amino acid residues and two-digit and four-digit HLA alleles were encoded as binary variables and phased by Beagle 3.0.4 imputation program (Browning & Browning, 2009) powered by SNP2HLA (to extract SNP genotypes located in the MHC region) method (Jia et al., 2013).

All genotype data from the two data sets were merged as a single data set after excluding the SNPs that were not present in both data sets (n = 3,175 SNPs in mainland GWAS data; n = 2,885 SNPs in Hong Kong GWAS data; n = 6,060 SNPs in both panels). The study subject was highly homogenous in a principal component (PC) analysis (Gibbs et al., 2003).

2.4 | Association analysis

For each phenotype, we assessed variant risk with a logistic-regression model assuming additive effects of the allele dosages in the log-odds scale. We defined HLA variants to include two- and four-digit biallelic classical HLA alleles, biallelic HLA amino acid polymorphisms for respective residues, multiallelic HLA amino acid polymorphisms and biallelic SNPs across the entire MHC region. To account for potential population substructure, we included the top ten PCs and an indicator variable for each data set as covariates when examined SLE association of the imputed dosage of each marker with minor allele frequency of ≥1% and imputation quality (PLINK R2) of ≥0.3 by logistic regression.

We also used a forward logistic regression model to find additional markers with independent SLE-risk effect by adding the identified markers as covariates for conditional analysis.

2.5 | Meta analysis

We used the inverse variance method for meta analysis, combining data from the two studies (Mainland and Hong Kong) for SNPs, alleles and amino acid with an imputation R2 score of ≥0.3 in two studies.

3 | RESULTS

3.1 | HLA Imputation and association testing

After imputation of SLE GWAS data for Chinese Han subjects (1,659 cases versus 3,398 controls) we obtained
genotypes for 10 HLA two-digit alleles, 13 HLA four-digit alleles, 173 HLA amino acid positions and 231 SNPs encoded by HLA genes of the class I and class II (p < 1.67 × 10^{-6}) from the genotyped MHC SNPs (29–34 Mb at chromosome 6). We set a study-wide significance threshold of p = 1.67 × 10^{-6} on the basis of the total number of imputed HLA variants (0.05/30,000).

3.2 | Risk of HLA-DQB1 at amino acid position 87 associated with SLE subjects

When testing the imputed HLA variants in the MHC region for association with SLE risk, we demonstrated the principal association with HLA-DQB1. We assessed the risk associated with both classical HLA alleles and HLA amino acid polymorphisms, and by a meta-analysis we found the most significant association was amino acid position 87 of HLA-DQB1 (P_{meta} = 7.81 × 10^{-17}, OR = 1.785; Figure 1a; Tables 1, S1).

3.3 | Independent HLA associations in HLA-DQB1*0301

We further investigated whether SLE risk was associated with other HLA genes independent of HLA-DQB1 amino acid polymorphisms. When conditioning on the most significant amino acid in HLA-DQB1 87, we detected significant independent associations at HLA-DQB1*0301 (P_{meta} = 6.91 × 10^{-15}, OR = 0.5508) (Figure 1b). After conditioning on HLA-DQB1*0301 and HLA-DQB1 amino acid position 87, no variants in the MHC region satisfied the study-wide significance threshold (p > 1.67 × 10^{-6}; Figure 1c; Table S2). These results suggested that polymorphisms in class II HLA genes, particularly HLA-DQB1, explain the majority of risk for SLE in Chinese Han population.

3.4 | HLA-DQB1 amino acid position 87 risks are shared between mainland and Hong Kong

After imputation in data set #2, we found that the effect size and direction of residue at HLA-DQB1 amino acid position 87 and HLA-DQB1*0301 in Hong Kong subjects were highly consistent with the results in mainland (p = 5.477 × 10^{-4} and 3.79 × 10^{-7}).

4 | DISCUSSION

Systemic Lupus Erythematosus (SLE) (OMIM 152700) is a polygenic disorder characterized by chronic and systemic inflammation and affecting multiple organs due to a loss of immune tolerance against self-antigens. The genetic heritability of SLE ranged from 44% to 66% (Lawrence, Martins, & Drake, 1987; Wang et al., 2007). To date, more than
60 susceptibility loci have been identified in genome wide and candidate gene association studies (Boackle, 2013). Association analysis of HLA genes at amino acid sites have facilitated fine-mapping efforts in immune-related diseases (Pereyra et al., 2010; Raychaudhuri et al., 2012). To identify potentially causal variation within HLA genes associated with Chinese SLE patients, we carried out an imputation based on the Han-MHC reference panel for classical HLA alleles as well as amino acid polymorphisms in mainland and Hong Kong Han SLE patients.

Our results support and refine previous findings of multiple signals in the MHC for population of Asia or European ancestry. By testing HLA alleles, amino acids, and SNPs at the same time, we also were able to pinpoint the amino acid position 11 and 13 of HLA-DR b as a significant signal in both mainland and Hong Kong Chinese Han populations (p = 2.91 x 10^-13 both), which was recognized as the major risk factor for SLE in Korean (Kim et al., 2014). This may suggest that genetic risk of the MHC region on SLE are generally shared within Asian populations to a certain extent (Kim et al., 2009, 2014). We note that those sites explain part of the variation in MHC-mediated risk for both populations, and that the residues confer similar directions and relative magnitude of risk. We also observe that allele HLA-DRB1*15:01 (p = 8.08 x 10^-11) and HLA-DQA1*0102 (p = 2.96 x 10^-10) in European populations (Morris et al., 2012, 2014) is generally concordant with the results presented here for Chinese Han populations.

HLA-DRB1 (MIM 142857) alleles are correlated with alleles of the class II loci HLA-DQA1 (MIM 146880) and HLA-DQB1 (MIM 604305) as a result of strong LD in the class II region (HLA-DR and HLA-DQ). However, amino acid in HLA-DQB1 have rarely been reported showing association with SLE. In our study amino acid position 87 of HLA-DQB1 has the most strongest association with SLE risk, which was firstly been reported till now. Furthermore, HLA-DQB1 amino acid position 87 and HLA-DQB1*0301 in Hong Kong individuals were highly consistent with mainland population. HLA genes encode cell surface proteins that display antigenic peptides to effector immune cells to regulate self-tolerance and downstream immune

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<th>Marker ID*</th>
<th>Positionb</th>
<th>Allelesc</th>
<th>Frequency [%]d</th>
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<th>Nearest gene</th>
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<td>1.576</td>
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</tr>
</tbody>
</table>

*Composite and low frequency markers (MAF < 1% in the entire sample set) are not shown.

bChromosome 6 positions for SNP markers according to genome build hg19.

cP/A: Present/Absent for classical HLA alleles and in the case that a specific amino acid is given in the Marker ID (see first column).

dFrequency and relative risk (OR) are given for the first allele denoted in the column “Alleles”.

### Table 1 Association results of the Top-20 associated markers after HLA imputation
diabetes-protective and -recessive HLA class II molecules. *Diabetologia*, 46(4), 496–503. https://doi.org/10.1007/s00125-003-1070-3


erythematosus in Asia: Where are we now? Genes and Immunity, 10(5), 421–432. https://doi.org/10.1038/gene.2009.24


SUPPORTING INFORMATION

Additional Supporting Information may be found in the supporting information tab for this article.

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