


RESEARCH ARTICLE

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The spectrum of association in HLA region with rheumatoid arthritis in a diverse Asian population: evidence from the MyEIRA case-control study

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Abstract

Background: Fine-mapping of human leukocyte antigen (*HLA*) region for rheumatoid arthritis (RA) risk factors has identified several *HLA* alleles and its corresponding amino acid residues as independent signals (i.e., *HLA-A*, *HLA-B*, *HLA-DPB1*, and *HLA-DQA1* genes), in addition to the well-established genetic factor in *HLA-DRB1* gene. However, this was mainly performed in the Caucasian and East Asian populations, and data from different Asian regions is less represented. We aimed to evaluate whether there are independent RA risk variants in both anti-citrullinated protein antibody (ACPA)-positive and ACPA-negative RA patients from the multi-ethnic Malaysian population, using the fine-mapping of *HLA* region strategy.

Methods: We imputed the classical *HLA* alleles, amino acids, and haplotypes using the Immunochip genotyping data of 1260 RA cases (i.e., 530 Malays, 259 Chinese, 412 Indians, and 59 mixed ethnicities) and 1571 controls (i.e., 981 Malays, 205 Chinese, 297 Indians, and 87 mixed ethnicities) from the Malaysian Epidemiological Investigation of Rheumatoid Arthritis (MyEIRA) population-based case-control study. Stepwise logistic regression was performed to identify the independent genetic risk factors for RA within the *HLA* region.

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Results: We confirmed that the HLA-DRB1 amino acid at position 11 with valine residue conferred the strongest risk effect for ACPA-positive RA (OR = 4.26, 95% CI = 3.30–5.49, $P_{GWAS} = 7.22 \times 10^{-29}$) in the Malays. Our study also revealed that HLA-DRB1 amino acid at position 96 with histidine residue was negatively associated with the risk of developing ACPA-positive RA in the Indians (OR = 0.48, 95% CI = 0.37–0.62, $P_{GWAS} = 2.58 \times 10^{-08}$). Interestingly, we observed that *HLA-DQB1*03:02* allele was inversely related to the risk of developing ACPA-positive RA in the Malays (OR = 0.17, 95% CI = 0.09–0.30, $P_{GWAS} = 1.60 \times 10^{-09}$). No association was observed between the HLA variants and risk of developing ACPA-negative RA in any of the three major ethnic groups in Malaysia.

Conclusions: Our results demonstrate that the RA-associated genetic factors in the multi-ethnic Malaysian population are similar to those in the Caucasian population, despite significant differences in the genetic architecture of *HLA* region across populations. A novel and distinct independent association between the *HLA-DQB1*03:02* allele and ACPA-positive RA was observed in the Malays. In common with the Caucasian population, there is little risk from HLA region for ACPA-negative RA.

Keywords: Rheumatoid arthritis, HLA amino acid residues, Risk variants, HLA fine-mapping, Multi-ethnic Malaysian population

Background

Extensive genetic studies during the last 40 years have demonstrated substantial contribution from the human leukocyte antigen DR beta chain 1 shared epitope (*HLA-DRB1* SE) alleles in RA pathogenesis, specifically for the subtype of RA that is positive for anti-citrullinated protein antibody (ACPA) [1–11]. However, there are differences in the allelic frequency of certain *HLA-DRB1* SE alleles across different populations. For instance, *HLA-DRB1*04:01* and *HLA-DRB1*04:04* alleles are common in RA patients with Caucasian ancestry, while *HLA-DRB1*04:05* allele is common in the Asian population [3, 6, 7, 9, 12–15]. It is also evident that the overall frequency of *HLA-DRB1* SE alleles in Asian populations is lower than in Caucasian populations, despite the similar RA prevalence between these populations. The reported population-specific risk allele *HLA-DRB1*09:01* in the Japanese and Korean populations suggests genetic factors other than *HLA-DRB1* SE are associated with risk of RA development [3, 16].

For the past decade, the cost-effective computational approach to infer HLA alleles using single nucleotide polymorphisms (SNP) genotypes within the HLA region has become a preferable method to study the HLA region in large-scale genetic association studies. This approach has also enabled the integration of functional data from large genomic data to understand the pathogenesis of RA [17, 18]. For instance, the polymorphic amino acid residues at position 11 (e.g., valine, HLA-DRB1 Val11 or leucine, HLA-DRB1 Leu11) within HLA-DRB1 protein explained most of the genetic risk of developing ACPA-positive RA [17, 19–21], instead of the amino acid residues previously defined at positions 71 and 74, the conserved amino acid region of the SE alleles [2]. In addition, independent association signals between single amino acid position within other HLA proteins and risk of developing

ACPA-positive RA demonstrated the importance of the HLA region in the pathogenesis of RA. For example, HLA-A amino acid residue at position 77 with asparagine residue (i.e., HLA-A Asn77), HLA-B amino acid residue at position 9 with aspartic acid (i.e., HLA-B Asp9), and HLA-DPB1 amino acid residue at position 9 with phenylalanine residue (i.e., HLA-DPB1 Phe9) are associated with risk of developing ACPA-positive RA [17, 21]. Furthermore, these predisposing HLA amino acid variants are located within the HLA molecules' peptide-binding grooves, suggesting the role of antigen binding and involvement in antigen presentation in the adaptive immune response. A recent study of a Han Chinese population reported that the aspartic acid at position 160 within the HLA-DQA1 protein (i.e., HLA-DQA1 Asp160) was associated with an increased risk of developing ACPA-positive RA, instead of the well-described *HLA-DRB1* alleles [22]. Comparative modeling analysis showed that the additional negative charge of HLA-DQA1 Asp160 enhances the interaction between the dimers of the major histocompatibility complex (MHC) class II molecules, which may lead to an increase in T cell activation [22].

These findings were mainly reported in the Caucasian, African, and East Asian populations, and there is very limited information about RA-associated polymorphic HLA amino acid residues in Southeast Asian populations. There is a need to expand the field of study to multiple genetically dissimilar populations to investigate the implication of HLA risk factors in the disease pathogenesis.

Thus, we fine-mapped the *HLA* region in the ACPA-positive and ACPA-negative RA subsets from the multi-ethnic Malaysian population [23]. We further investigated the association between different HLA-DRB1 amino acid variants and compared the HLA-DRB1 amino acid haplotypes in different ethnic groups for risk of developing different subsets of RA.

Methods

Study design and study population

This study utilized data from the Malaysian Epidemiological Investigation of Rheumatoid Arthritis (MyEIRA), a large population-based case-control study of RA conducted in the multi-ethnic Malaysian population. The study design of MyEIRA has been described elsewhere [9, 24]. Briefly, this study analyzed data from 1260 patients with early RA (i.e., 530 Malays, 259 Chinese, 412 Indians, and 59 mixed ethnicities), and 1571 matched controls (i.e., 981 Malays, 206 Chinese, 297 Indians, and 87 with mixed ethnicities).

The RA cases were identified from nine rheumatology clinics throughout Peninsular Malaysia between 2005 and 2009. All RA cases were diagnosed according to the 1987 revised American College of Rheumatology (ACR) classification of rheumatoid arthritis criteria by rheumatologists. For each RA case, a control was randomly selected from the general population, matched for age, sex, and residential area. All study subjects were unrelated and ethnicity background was self-reported, based on questions about ancestry.

Anti-citrullinated protein antibody measurement

The presence of ACPA in all individuals was assessed using anti-cyclic citrullinated peptide second-generation (anti-CCP2) ELISA kits (Immunoscan RA, Malmö, Sweden). Samples with results > 25 AU/mL were defined as ACPA-positive [9].

HLA genotyping

The experimental classical *HLA* genotyping for *HLA-A*, *HLA-B*, *HLA-C*, *HLA-DRB1*, and *HLA-DQB1* genes was performed previously and described elsewhere [9, 25]. In brief, the *HLA* genotyping was performed for all DNA samples using the polymerase chain reaction and sequence-specific oligonucleotide probe hybridization (PCR-SSO) method (LABType® *HLA* test kits, One Lambda Inc., CA, USA) on the Luminex Multi-Analyte Profiling System (xMAP, Luminex Corporation, TX,

USA). The *HLA* typing assignment was accomplished using the *HLA* Fusion software (version 1.3.0) provided by the manufacturer (One Lambda Inc., CA, USA).

Dense SNP genotyping and quality controls

All individuals were genotyped using the Illumina iSelect HD custom genotyping array designed by the ImmunoChip Consortium (ImmunoChip, Illumina, Inc., San Diego, CA, USA). The ImmunoChip array was custom-designed with a dense coverage of *HLA* region to perform deep replication of major autoimmune and inflammatory diseases, including RA [23, 26]. The genotyping quality control (QC) was performed using PLINK v1.07 software [27]. The SNPs with call rate less than 99%, minor allele frequency (MAF) less than 0.01, and with significant departure from Hardy-Weinberg equilibrium (HWE) ($p < 0.001$), in both the RA cases and control groups, were excluded. Individuals with missing genotyping rate higher than 10% were also excluded. Then, a total of 25 individuals from the RA group (i.e., redundant RA and non-RA) were removed, followed by removal of a further 11 individuals (i.e., 6 RA cases and 5 matched normal controls) with missing genotyping rate > 10%, from the subsequent data analysis. A total of 113,576 SNPs in 2795 individuals (i.e., 1229 RA cases and 1566 controls) remained after QC. The individuals with mixed ethnicity parentage background were excluded from further analysis. Thus, the association testing was restricted to study subjects whose parents both came from the same ethnic group, giving a total of 1170 RA and 1479 controls for analysis after QC. The baseline demographic characteristics of the RA cases and controls are shown in Table 1.

Imputation of classical *HLA* alleles and polymorphic amino acids residues

A total of 6152 SNPs between positions 29 and 34 Mb in the *HLA* region on chromosome 6 (GRCh37) were extracted from the post-QC ImmunoChip dataset. Using the extracted SNP genotypes from the *HLA* region, we

Table 1 Demographic characteristics of patients with rheumatoid arthritis and controls in MyEIRA case-control study

Characteristics	All		Ethnicity					
			Malay		Chinese		Indian	
	RA cases* (n = 1229)	Controls* (n = 1566)	RA cases* (n = 514)	Controls* (n = 981)	RA cases* (n = 252)	Controls* (n = 204)	RA cases* (n = 404)	Controls* (n = 294)
Mean age (years old, SD)	48.11 ± 11.61	47.22 ± 11.37	46.18 ± 11.77	46.36 ± 11.40	52.27 ± 11.21	50.96 ± 11.31	47.92 ± 10.81	48.24 ± 10.58
Female (%)	85.8	85	86.2	85.7	80.9	82.8	87.3	82.3
ACPA positivity (%)	64.4	2.4	60.5	2.4	66.2	3.4	67.1	2.1
<i>HLA-DRB1</i> SE positivity (%)	40.1	16.2	35.6	12.9	36.5	12.3	48.3	29.9

All represent the total number of RA cases and matched controls for the Malay, Chinese, Indian and other/mixed ethnicities in this study. MyEIRA Malaysian Epidemiological Investigation of Rheumatoid Arthritis, RA rheumatoid arthritis, SD standard deviation, ACPA anti-citrullinated protein antibody, *HLA-DRB1* SE *HLA* DR beta 1 shared epitope. *The number of individuals for the RA cases and controls was based on the number of individuals passing the dense SNP genotyping dataset quality control

imputed the HLA variants (i.e., classical 2-digit and 4-digit *HLA* alleles, and polymorphic amino acid residues of the *HLA* genes), along with the SNPs from the Pan-Asian reference panel [18, 19]. The Pan-Asian reference panel comprised 530 unrelated individuals of Asian descent: i.e., Han Chinese ($n = 247$, 46.6%), Malays ($n = 120$, 22.6%), Tamil Indians ($n = 119$, 22.4%), and Japanese ($n = 44$, 8.3%). The reference panel included a total of 6173 SNPs associated to 94 classical 2-digit *HLA* alleles, 179 classical 4-digit *HLA* alleles, and 1799 polymorphic amino acid positions [19, 28]. All the RA cases and controls were imputed together using the SNP2HLA software [18].

HLA allele imputation accuracy assessment

We assessed the imputation accuracy for each imputed classical *HLA* allele in *HLA-A*, *HLA-B*, *HLA-C*, *HLA-DRB1*, and *HLA-DQB1* genes using experimental and imputed classical *HLA* genotype datasets from the normal controls with Malays, Chinese, and Indians. In brief, concordance rate was defined as the count of matched imputed classical *HLA* allele to the experimental classical *HLA* allele at the individual level, divided by the total count of observed experimental classical *HLA* allele within the studied population. Imputation accuracy assessment only considers individuals with available data for both experimental and imputed *HLA* genotypes. The *HLA* alleles' distributions and their allelic frequencies vary in different populations/ethnic groups, so we further assessed the imputation accuracy in the three ethnic groups, i.e., Malays, Chinese, and Indians. Imputation accuracy with concordance rate above 90% was considered as high imputation accuracy threshold in this study.

Association analysis of HLA alleles and amino acid polymorphisms

Referring to the data analysis described elsewhere, the logistic regression model was applied to test for the association between the imputed HLA variants and risk of developing different subsets of RA, separately in the Malay, Chinese, and Indian ethnic groups, with adjustment for age and sex [17, 19, 29]. The imputed HLA variants were defined by including the biallelic SNPs, classical 2-digit *HLA* alleles, classical 4-digit *HLA* alleles, and polymorphic HLA amino acid residues [17, 19, 29]. The analyses were conducted in PLINK v1.07 software [27]. The significance threshold of p value (P_{GWAS}) was less than 5×10^{-8} in this study.

We implemented a stepwise logistic regression conditioned by the most associated variants, to search for the independent effects across the *HLA* region. All variables (i.e., imputed HLA variants) were systematically removed/added to obtain the best fit model based on the P_{GWAS} threshold. The Akaike information criterion (ΔAIC) and

the improvement in the Bayesian information criterions (ΔBIC) were also considered to assess the best fit model. A modified version of a public Python 3.0 script (<http://trevor-smith.github.io/stepwise-post/>), which uses the Statsmodels module [30], was used in this analysis.

HLA amino acid haplotype analysis

A group of RA-related classical *HLA-DRB1* alleles encoding a conserved amino acid sequence ($^{70}QRRRAA^{74}$ or $^{70}KRRRAA^{74}$ or $^{70}RRRAA^{74}$) at positions 70 to 74 in the third hypervariable region of the first domain of *DRB1* was defined as shared epitope (SE) [2]. The *HLA-DRB1* SE alleles are the most established genetic risk factors for RA [2, 9, 31]. Nevertheless, the recent studies demonstrated that polymorphic *HLA-DRB1* amino acid residues at positions 11 and 13 were the top association signals for risk of RA, instead of positions 70–74 [17, 19]. Hence, we aimed to replicate the investigation of *HLA-DRB1* amino acid haplotypes and risk for ACPA-positive RA in the Caucasian and East Asian populations [17, 19], for the Malays, Chinese, and Indians.

We constructed the haplotypes manually based on the RA risk *HLA-DRB1* haplotype model (i.e., defined by the polymorphic amino acid residues at positions 11, 13, 71, and 74), by filtering the subsets of *HLA-DRB1* 11-13-71-74 haplotypes in PLINK v1.07 software. First, we assessed the association between these *HLA-DRB1* amino acid haplotypes and risk for ACPA-positive RA in all three ethnic groups. Then, we observed the risk effect (expressed as odds ratio, OR) between the published data and findings from the Malay, Chinese, and Indian ethnic groups.

Meta-analysis and comparative analysis with published data

To test the generalizability of the polymorphic amino acid residues at position 11 within the *HLA-DRB1* protein and risk of developing ACPA-positive RA in the Malay, Chinese, and Indian ethnic groups, we performed a meta-analysis using the Mantel-Haenszel method, with the random-effect model by means of cumulative OR with 95% confidence interval (95% CI). The heterogeneity between the studied ethnic groups was assessed using the Cochran Q-statistic ($P < 0.10$ considered significant). In addition, the I^2 metric [$I^2 = (Q - df)/Q$] was used to describe the percentage of variation across the different ethnic groups due to heterogeneity. I^2 values of 25%, 50%, and 75% were considered as low, moderate, and high estimates, respectively. All analyses were performed in the PLINK v1.07 and Review Manager v5.3 (Copenhagen, The Nordic Cochrane Centre, The Cochrane Collaboration, 2014) software.

We compared the findings from this study with the published RA-associated genetic variants within the

HLA region from different populations/ethnic groups (i.e., Caucasian, East Asian, African, and Han Chinese) to investigate the spectrum of association in the HLA region with risk of developing RA [17, 19–22]. Here, we restricted the selection of published RA-associated HLA variants to those computationally imputed from dense SNP genotypes within the HLA region.

Results

Imputed HLA variants and imputation accuracy assessment

We imputed a total of 3239 markers comprising 90 classical 2-digit HLA alleles, 175 classical 4-digit HLA alleles, 1799 specific HLA amino acid positions, and 1175 SNPs from the Pan-Asian reference panel. Our data demonstrated the overall concordance rate of the classical 2-digit HLA alleles satisfied the suggested concordance rate threshold of 90% for all five HLA genes, while decreased overall rates (ranged between 71.5 and 85.7%) were observed at 4-digit resolution (supplementary Table 1). Notably, the decreased overall concordance rates were attributed to the increased polymorphisms detected in these HLA genes. We further observed the concordance rates varied among the imputed classical HLA alleles at 2-digit and 4-digit resolutions for all HLA genes, where the variations were influenced by the distribution of the common/rare HLA alleles and its allelic frequency varies across different ethnic groups (supplementary Tables 2 and 3).

HLA-DRB1 variants associated with risk of developing RA

The logistic regression analysis demonstrated that the genome-wide significant threshold (P_{GWAS}) for association analysis was satisfied by 15 classical HLA class II alleles, which were located in HLA-DRB1 ($n = 6$), HLA-DQA1 ($n = 4$), and HLA-DQB1 ($n = 5$) genes; 74 amino acid polymorphisms (47.3% in the HLA-DRB1 protein, 28.4% in the HLA-DQB1 protein, 20.3% in the HLA-DQA1 protein, and only 4.1% in the HLA Class I protein); and 128 SNP variants (supplementary Table 4). However, the distribution of these identified significant risk variants varied across the Malay, Chinese, and Indian ethnic groups, with the majority observed in the Malays.

In the Malay ethnic group, the most significant association across all variants tested was observed at HLA-DRB1 Val11 (OR = 4.26, 95% CI = 3.30–5.49, $P_{GWAS} = 7.22 \times 10^{-29}$), followed by HLA-DRB1 amino acid at position 120 with asparagine residue (i.e., HLA-DRB1 Asn120) (OR = 4.23, 95% CI = 3.28–5.45, $P_{GWAS} = 8.59 \times 10^{-29}$), which is in tight linkage disequilibrium (LD, $D' = 1.00$) with HLA-DRB1 Val11 (Table 2 and supplementary Table 5). Further peptide alignment analysis of the HLA-DRB1 protein revealed that the HLA-DRB1 Val11 and HLA-DRB1 Asn120 are exclusive characteristics for all the HLA-DRB1*04 and HLA-DRB1*10 alleles, indicating HLA-DRB1 Asn120 is not an independent risk factor for developing ACPA-positive RA among the Malay patients (online HLA alignment database <https://www.ebi.ac.uk/cgi-bin/imgt/hla/align.cgi>). Our observations implied that we have convincingly replicated the previous

Table 2 Top association signals between HLA variants and risk of developing RA in the Malaysian population

Ethnicity	HLA variant	Position	Amino acid residue	Allele frequency		OR	95% CI	P
				RA cases	Controls			
Overall RA								
All	HLA-DRB1 position 120	32,657,518	Asparagine	0.25	0.12	2.46	2.13–2.84	$1.55 \times 10^{-34*}$
Malay	HLA-DRB1 position 11	32,660,115	Valine	0.21	0.09	2.86	2.28–3.58	$4.11 \times 10^{-20*}$
Chinese	HLA-DRB1*04:05	32,660,042	–	0.13	0.04	3.47	2.00–6.03	9.49×10^{-06}
Indian	HLA-DRB1 position 96	32,657,590	Histidine	0.42	0.53	0.64	0.51–0.79	6.16×10^{-05}
ACPA-positive RA								
All	HLA-DRB1 position 120	32,657,518	Asparagine	0.31	0.12	3.34	2.84–3.92	$5.96 \times 10^{-49*}$
Malay	HLA-DRB1 position 11	32,660,115	Valine	0.28	0.09	4.26	3.30–5.49	$7.22 \times 10^{-29*}$
Chinese	HLA-DRB1*04:05	32,660,042	–	0.18	0.04	5.22	2.95–9.25	$1.52 \times 10^{-08*}$
Indian	HLA-DRB1 position 96	32,657,590	Histidine	0.37	0.53	0.48	0.37–0.62	$2.58 \times 10^{-08*}$
ACPA-negative RA								
All	HLA-DRB1 position 60	32,659,968	Serine	0.15	0.12	0.63	0.54–0.75	7.09×10^{-08}
Malay	HLA-DRB1 position 60	32,659,968	Serine	0.32	0.42	0.64	0.52–0.81	1.40×10^{-04}
Chinese	HLA-B*27:04	31,431,272	–	0.06	0.02	4.40	1.55–12.53	5.51×10^{-03}
Indian	HLA-B position 12	31,432,680	Valine	0.36	0.27	1.49	1.09–2.04	1.00×10^{-02}

The table shows the top association signals between HLA variants and risk of developing ACPA-positive and ACPA-negative RA in the Malay, Chinese, and Indian ethnic groups. All combined group of individuals with Malay, Chinese, Indian, and others/mixed ethnicities, RA rheumatoid arthritis, ACPA anti-citrullinated protein antibody, HLA human leukocyte antigen, DRB1 DR beta chain 1, OR odds ratio, 95% CI 95% confidence interval, P p value. *Achieved genome-wide association threshold of $P_{GWAS} < 5 \times 10^{-8}$

published data showing the association of the *HLA-DRB1*04:05* allele (i.e., corresponding to HLA-DRB1 Val11 and HLA-DRB1 Asn120) with increased risk of ACPA-positive RA in the Malay ethnic group [9].

Our findings in the Chinese ethnic group showed that *HLA-DRB1*04:05* allele was the top association signal for risk of ACPA-positive RA (OR = 5.22, 95% CI = 2.95–9.25, $P_{GWAS} = 1.52 \times 10^{-08}$) (Table 2), in agreement with the previously published data using the experimental classical HLA genotype dataset [9]. Furthermore, we observed the association between HLA-DRB1 Asn120 and risk of ACPA-positive RA in the Chinese ethnic group; however, the signal was below the suggested P_{GWAS} threshold (OR = 3.05, 95% CI = 2.03–4.58, $P_{GWAS} = 8.43 \times 10^{-08}$). Moreover, *HLA-DRB1*04:05* allele is one of the corresponding alleles to HLA-DRB1 Val11 and HLA-DRB1 Asn120, which are in tight LD ($D' = 1.00$), based on the online database of HLA peptide sequence (supplementary Table 5, online HLA alignment database <https://www.ebi.ac.uk/cgi-bin/ipd/imgt/hla/align.cgi>). In view of the evidence for HLA-DRB1 Val11 as a common risk factor for ACPA-positive RA across different populations [17, 19, 20, 22], we tested the association between this variant and risk of ACPA-positive RA in the Chinese ethnic group. Our finding confirmed the increased risk of ACPA-positive RA in the Chinese ethnic group (OR = 2.87, 95% CI = 1.91–4.30, $P_{GWAS} = 3.63 \times 10^{-7}$), although this did not reach genome-wide significance (supplementary Table 5).

Further stratification analysis by ethnicity revealed the strongest association signal at amino acid position 96 within HLA-DRB1 peptide with histidine residue (i.e., HLA-DRB1 His96) among Indian patients with ACPA-positive RA (OR = 0.48, 95% CI = 0.37–0.62, $P_{GWAS} = 2.58 \times 10^{-08}$) (Table 2). The HLA-DRB1 peptide alignment showed that HLA-DRB1 His96 corresponded to specific alleles from *HLA-DRB1*03*/07*/08*/09*/11*/12/*

**13*/14* allele groups (online HLA alignment database <https://www.ebi.ac.uk/cgi-bin/ipd/imgt/hla/align.cgi>). Of these *HLA-DRB1* alleles, the *HLA-DRB1*13* allele group was inversely associated with the risk of ACPA-positive RA in the Caucasian, Japanese, and Indian Tamil populations in previous studies [32–34]. Although the commonly shared variant of HLA-DRB1 Val11 also increased the risk of ACPA-positive RA among the Indian patients, this association did not reach the genome-wide significant threshold (OR = 1.99, 95% CI = 1.52–2.61, $P_{GWAS} = 6.60 \times 10^{-07}$) (supplementary Table 5).

We did not observe any significant association between the imputed HLA variants and risk of developing ACPA-negative RA in any of the three major ethnic groups (data not shown).

Risk factor independent from HLA-DRB1 in the ACPA-positive RA subset

To look for independent effects across the HLA region, we conducted a stepwise logistic regression. Conditioning by the most associated risk variant, i.e., HLA-DRB1 Val11, with ACPA-positive RA in the Malay ethnic group revealed an inverse association of *HLA-DQB1*03:02* allele with risk of developing ACPA-positive RA (OR = 0.17, 95% CI = 0.09–0.30, $P_{GWAS} = 1.60 \times 10^{-09}$) (Fig. 1). No further independent risk variants were detected within the HLA region for ACPA-positive RA in the Malay ethnic group. This finding was confirmed by using the experimental classical *HLA* genotype dataset that demonstrated an inverse association between *HLA-DQB1*03:02* allele and risk of ACPA-positive RA (OR = 0.27, 95% CI = 0.17–0.45, $p = 2.30 \times 10^{-07}$) (supplementary Table 6).

“The stepwise logistic regression demonstrated that the top association signals for the Chinese and Indian ethnic groups were *HLA-DRB1*04:05* allele (OR = 3.99, 95% CI = 2.22–7.18, $p = 3.90 \times 10^{-06}$) and HLA-DRB1

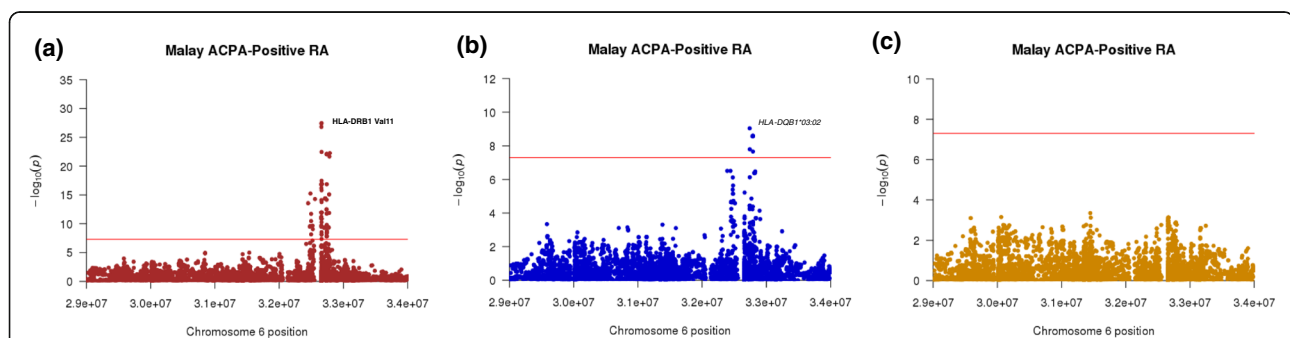


Fig. 1 Association plots of the tested variants in the HLA region to ACPA-positive RA in Malays. Legends: **a** Regional plot of stepwise logistic regression in ACPA-positive Malay RA patients, HLA-DRB1 Val11 mapped as the strongest association signal ($P_{GWAS} = 2.4 \times 10^{-35}$). **b** Conditioning of HLA-DRB1 Val11, *HLA-DQB1*03:02* alleles ($P_{GWAS} = 1.57 \times 10^{-9}$) were mapped as the second independent HLA risk variants with decreased risk of developing ACPA-positive RA. **c** No independent variants observed from conditioning the two most associated HLA variants, i.e., HLA-DRB1 Val11 and *HLA-DQB1*03:02* allele. The red line represents the suggested significant threshold, i.e., genome-wide significant threshold of $P_{GWAS} < 5 \times 10^{-8}$

His96 (OR = 0.40, 95% CI = 0.30–0.52, $p = 5.25 \times 10^{-08}$), respectively. However, these two variants did not satisfy the genome-wide significant threshold (supplementary figure 1). Conditioning on these top association signals showed that no further independent HLA risk variants were detected for ACPA-positive RA in the Chinese and Indian ethnic groups.”

Comparative analysis for the independent effects of HLA amino acid variants and risk of ACPA-positive RA across different populations

We compared the published independent RA-associated polymorphic HLA amino acid positions across different populations/ethnic groups and the data is presented in Table 3. The HLA-DRB1 Val11 was the most common HLA amino acid variant significantly associated ($P_{Gwas} < 5 \times 10^{-08}$) with increased risk of ACPA-positive RA in all the studied populations included in this study

(Table 3), and this association was validated in our study among the Malay ethnic group. Within the same HLA protein, amino acid position 13 with histidine residue was associated with increased risk for ACPA-positive RA in the East Asian and African populations; it was, however, in tight LD with HLA-DRB1 Val11. Furthermore, different amino acid positions, i.e., positions 37, 57, and 74, were reported as RA-associated genetic variants in the ACPA-positive RA from the East Asian population (Table 3). We did not observe the RA-associated polymorphic HLA-DRB1 amino acid at positions 13, 37, 57, and 74 associated with the risk of developing ACPA-positive RA in our study population. However, the independent effect of HLA-DRB1 His96 associated with decreased risk for ACPA-positive RA in the Malaysian Indian patients was not reported in any of the published data from these studied populations.

This observation supported the genetic association of the HLA region to RA and that this is commonly

Table 3 Independent specific amino acid residues associated with risk of developing ACPA-positive RA in different populations

Region	Population	RA cases	Controls	HLA locus	Amino acid position	Residue	OR	P	χ^2	$P_{omnibus}$	Ref	
Europe	Caucasian	5018	14,974	HLA-DRB1	11	Valine	3.80	–	2260.0	1.0×10^{-581}	[17]	
				HLA-B	9	Aspartic acid	2.12	–	166.0	2.0×10^{-37}		
				HLA-DPB1	9	Phenylalanine	1.40	–	93.3	1.0×10^{-20}		
	Caucasian	7279	15,870	HLA-DRB1	11	Valine	–	–	3551.5	1.0×10^{-692}	[21]	
				HLA-B	9	Aspartic acid	1.93	1.0×10^{-36}	160.2	–		
				HLA-DPB1	9	Phenylalanine	1.31	1.0×10^{-19}	82.6	–		
East Asia	Korean	616	675	HLA-DRB1	11	–	–	–	–	6.1×10^{-36}	[17]	
				HLA-DRB1	13	–	–	–	–	3.1×10^{-36}		
	Korean and Chinese	2782	4315	HLA-DRB1	13	Histidine	2.03	–	–	–	6.9×10^{-135}	[19]
				HLA-DRB1	57	–	–	–	–	2.2×10^{-33}		
				HLA-DRB1	74	–	–	–	–	1.1×10^{-08}		
				HLA-DRB1	11	–	–	–	–	1.7×10^{-129}		
				HLA-B	9	Aspartic acid	4.21	3.8×10^{-06}	21.4	–		
	Han Chinese	961	1812	HLA-DQA1	160	Aspartic acid	2.29	6.2×10^{-36}	156.6	–		[22]
				HLA-DRB1	11	Valine	1.79	2.1×10^{-13}	53.9	–		
HLA-DRB1				37	Asparagine	0.49	5.8×10^{-16}	65.5	–			
Africa	African	266	362	HLA-DRB1	11	Valine	5.1	3.4×10^{-26}	112.1	–	[20]	
				HLA-DRB1	13	Histidine	6.1	1.2×10^{-27}	118.7	–		
Malaysian*	Malay	311	981	HLA-DRB1	11	Valine	4.26	7.2×10^{-29}	121.1	–	–	
	Chinese	167	204	HLA-DRB1	11	Valine	2.87	3.6×10^{-07}	25.9	–	–	
	Indian	195	294	HLA-DRB1	96	Histidine	0.48	2.2×10^{-08}	31.3	–	–	

The table compares the reported independent association between HLA amino acid variants and the risk of developing ACPA-positive RA in different populations. ACPA anti-citrullinated protein antibody, HLA human leukocyte antigen, DRB1 DR beta 1, DPB1 DP beta 1, DQA1 DQ alpha 1, DQB1 DQ beta 1, OR odds ratio, P p value; χ^2 chi-square, $P_{omnibus}$ omnibus p value. *The Malaysian population comprising different ethnic groups: the Malay (i.e., predominant southeast Asian ethnic group) [25], Chinese (i.e., Han Chinese descendants of immigrants who arrived during the nineteenth and early twentieth century from Southern China) [35], and Indian (Tamil descendants of immigrants who arrived during the nineteenth and early twentieth century from Southern India) [36]

attributed to *HLA-DRB1* genes. Furthermore, the observed risk effects of the different amino acids from the same *HLA-DRB1* protein suggested that while some may promote the pathogenic process in RA, others may counteract the process.

We further observed that the polymorphic HLA amino acid positions independent of *HLA-DRB1* gene were associated with the risk of developing ACPA-positive RA in a population-specific manner. For instance, HLA-A Asn77, HLA-B Asp9, and HLA-DPB1 Phe9 were reported as RA-associated genetic variants in the Caucasian populations, while *HLA-DRB1* His13 was RA-associated in the East Asian and African populations. More recently, the *HLA-DQA1* Asp160 was reported in Han Chinese to be associated with an increased risk of ACPA-positive RA. However, we did not observe any association for these amino acid variants with ACPA-positive RA in our study population with Malay, Chinese, or Indian origins.

To summarize, these predisposing HLA-specific amino acid positions may exhibit shared-genetic component or population-specific risk signals, suggesting the existence of ethnogenetic heterogeneity in the RA population.

HLA-DRB1 amino acid haplotypes as risk factors for ACPA-positive RA

Of the 16 possible *HLA-DRB1* amino acid haplotypes at positions 11, 13, 71, and 74 [17], we observed only 10, 12, and 12 haplotypes, respectively, in Malay, Chinese, and Indian ethnic groups to be associated with ACPA-positive RA (Table 4). Our findings revealed that the Val¹¹-His¹³-Arg⁷¹-Ala⁷⁴ haplotype was strongly associated with risk of ACPA-positive RA in the Malay (OR = 5.28, 95% CI = 3.06–9.09, $p = 1.22 \times 10^{-09}$), Chinese (OR = 10.33, 95% CI = 4.39–24.31, $p = 9.81 \times 10^{-09}$), and Indian (OR = 3.84, 95% CI = 3.75–4.75, $p = 0.03$) populations (Table 4). Meanwhile, we observed the Val¹¹-Phe¹³-Arg⁷¹-Ala⁷⁴ haplotype was associated with increased risk of ACPA-positive RA in the Malays (OR = 4.35, 95% CI = 2.27–8.32, $p = 9.78 \times 10^{-06}$) and Indians (OR = 2.00, 95% CI = 1.10–3.68, $p = 0.03$), but not in the Chinese. Interestingly, while Ser¹¹-Ser¹³-Glx⁷¹-Ala⁷⁴ conferred significant risk for ACPA-positive RA among the Chinese (OR = 12.91, 95% CI = 2.55–65.34, $p = 6.98 \times 10^{-04}$), it demonstrated an inverse association to ACPA-positive RA in the Indian population (OR = 0.40, 95% CI = 0.18–0.86, $p = 0.03$).

Comparing our findings from the multi-ethnic Malaysian population with the published data from other populations with European and East Asian origins, the Val11-His13-Arg71-Ala74 was the most significant and commonly shared risk factor among the European and Asian populations (Table 4). The decreased risk of ACPA-positive RA associated with the Ser¹¹-Ser¹³-Glx⁷¹-Ala⁷⁴ haplotype observed in the European and East Asian populations was consistently replicated in the

Malaysian Indian ethnic group. In contrast, this haplotype conferred risk for ACPA-positive RA in the Malaysian Chinese ethnic group. Notably, this haplotype is encoded by *HLA-DRB1*13:01* and *HLA-DRB1*13:03* alleles. It has been previously reported that the *HLA-DRB1*13* allele has a dual role: as genetic modulator of ACPA positivity, whereby it was inversely associated with risk of ACPA-positive RA; but also, in combination with *HLA-DRB1*03*, it decreased the risk of ACPA-negative RA [36]. Our observation in the Malaysian Chinese ethnic group was not in line with the inverse association to ACPA-positive reported in the Caucasian and East Asian populations, suggesting different immune reactions may occur in RA with different ethnicity/population backgrounds.

Amino acid polymorphisms at position 11 within HLA-DRB1 protein and risk of RA

We investigated the frequency of the polymorphic amino acid residues (i.e., valine, serine, proline, leucine, glycine, and aspartic acid) at position 11 in the *HLA-DRB1* protein of the Malaysian population with Malay, Chinese, and Indian origins and further compared these frequencies with the published data from Caucasian and East Asian populations [19]. Our data demonstrated that while the frequency of valine residue was higher in RA cases as compared to the normal control group in all the populations, the frequency of serine residue was lower in the RA cases in comparison with the normal controls (Fig. 2). Interestingly, leucine residue, which encodes the classical *HLA-DRB1*01* alleles, was commonly found among the individuals of European ancestry (>10% in both RA cases and control group), but was found in less than 5% of the Malay and Indian ethnic groups, and was absent in the Chinese RA cases and controls. It is noteworthy that the aspartic acid residue was commonly found in the Chinese individuals. This amino acid residue corresponds to the classical *HLA-DRB1*09*, an allele which was reported as a risk factor for RA development, independent of the *HLA-DRB1 SE* alleles [19]. The frequencies of proline and glycine residues, which encode the classical *HLA-DRB1*15* and *HLA-DRB1*07* alleles respectively, were comparable between RA cases and control group for all populations (Fig. 2).

Next, we performed meta-analyses to investigate the generalizability of the effect of the polymorphic *HLA-DRB1* amino acid residues at position 11 on the risk for ACPA-positive RA in the Malay, Chinese, and Indian ethnic groups. Our finding demonstrated significant cumulative OR of the *HLA-DRB1* Val11 for risk of ACPA-positive RA (OR_{cumulative} 2.86–2.90, $p < 0.0001$); however, we observed high heterogeneity within studies ($I^2 = 91\%$) (supplementary Figure 2a). Interestingly, we observed a decreased risk of developing ACPA-positive

Table 4 HLA-DRB1 amino acid haplotypes and risk of ACPA-positive RA in different populations

HLA-DRB1 amino acid at position			Malay [#]		Chinese [#]		Indian [#]		European ^a		European ^b		East Asian ^c		Classical HLA-DRB1 alleles	
11	13	71	74	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	
Pro	Arg	Ala	Ala	Ref		Ref		Ref		Ref		Ref		Ref		*15:01, *15:02, *15:04, *16:02
Val	His	Arg	Ala	5.28	3.06–9.09	10.33	4.39–24.31	3.84	1.02–14.44	4.22	3.75–4.75	3.63	3.29–4.01	3.02	2.62–3.48	*04:04, *04:05, *04:10
Val	Phe	Arg	Ala	4.35	2.27–8.32	-	-	2.00	1.10–3.68	-	-	4.65	3.80–5.70	2.83	2.22–3.61	*10:01
Ser	Ser	Glx	Ala	0.36	0.11–1.22	12.91	2.55–65.34	0.40	0.18–0.86	0.59	0.51–0.68	0.60	0.54–0.67	0.6	0.50–0.72	*13:01, *13:02
Ser	Ser	Lys	Arg	0.64	0.30–1.37	0.81	0.27–2.40	0.82	0.37–1.78	0.63	0.54–0.73	0.67	0.60–0.76	0.71	0.53–0.96	*03:01
Gly	Tyr	Arg	Gln	0.78	0.46–1.34	1.61	0.28–9.41	0.74	0.42–1.30	0.91	0.80–1.03	0.92	0.83–1.02	0.9	0.75–1.08	*07:01
Ser	Gly	Arg	Ala	0.83	0.57–1.22	1.67	0.76–3.66	1.28	0.50–3.30	0.88	0.77–1.00	1.04	0.86–1.25	1.12	0.95–1.32	*12:01, *12:02, *12:03
Ser	Ser	Arg	Ala	0.72	0.29–1.81	2.69	1.02–7.07	0.56	0.22–1.41	-	-	0.76	0.67–0.86	0.83	0.68–1.02	*11:01, *11:05, *13:12
Val	His	Arg	Glx	0.72	0.29–1.81	1.88	0.66–5.38	1.09	0.57–2.07	1.65	1.24–2.19	1.29	1.06–1.57	0.95	0.79–1.13	*04:03, *04:06
Ser	Gly	Arg	Glx	1.08	0.49–2.39	1.21	0.30–4.96	0.72	0.38–1.37	-	-	0.49	0.26–0.91	0.56	0.42–0.74	*14:04
Ser	Gly	Arg	Leu	-	-	1.41	0.51–3.87	0.87	0.25–3.00	0.71	0.57–0.89	0.83	0.70–0.98	0.85	0.72–1.01	*08:01, *08:02, *08:03, *08:09
Ser	Ser	Arg	Glx	-	-	1.34	0.43–4.24	-	-	0.84	0.67–1.05	0.77	0.64–0.94	0.77	0.60–0.99	*14:01, *14:05, *14:07
Ser	Ser	Arg	Leu	-	-	1.41	0.51–3.87	-	-	-	-	-	-	-	-	*14:03
Val	His	Lys	Ala	-	-	-	-	1.92	0.67–5.51	4.44	4.02–4.91	4.03	3.72–4.37	3.63	2.63–5.00	*04:01
Leu	Phe	Arg	Ala	-	-	-	-	-	-	2.17	1.94–2.42	2.11	1.94–2.31	1.51	1.26–1.80	*01:01, *01:02
Asp	Phe	Arg	Glx	-	-	-	-	-	-	1.65	1.29–2.10	1.82	1.52–2.18	1.8	1.56–2.09	*09:01
Pro	Arg	Arg	Ala	-	-	-	-	-	-	2.04	1.59–2.62	1.58	1.26–1.99	1.21	0.85–1.73	*16:01, *16:02
Val	His	Glx	Ala	-	-	-	-	-	-	1.43	1.04–1.96	1.03	0.71–1.50	-	-	*04:02, *04:37
Ser	Ser	Lys	Ala	-	-	-	-	-	-	1.04	0.76–1.41	0.87	0.66–1.14	-	-	*13:03
Leu	Phe	Glx	Ala	-	-	-	-	-	-	0.73	0.42–1.27	0.71	0.55–0.93	-	-	*01:03

ACPA anti-citrullinated protein antibody. HLA human leukocyte antigen. DRB1 DR beta chain 1. OR odds ratio. 95% CI 95% confidence interval. Amino acid abbreviations: Pro proline, Arg arginine, Ala alanine, Val valine, His histidine, Phe phenylalanine, Ser serine, Glx glutamic acid/glutamine, Gly glycine, Tyr tyrosine, Gln glutamine, Leu leucine, Lys lysine. [#]The individuals with Malay, Chinese, and Indian ethnicity recruited for Malaysian Epidemiological Investigation of Rheumatoid Arthritis (MEIRA) population-based case-control study. ^aPublished data retrieved from Raychaudhuri et al. [17]. ^bPublished data retrieved from Han et al. (2014) [21]. ^cPublished data retrieved from Okada et al. (2014) [37].

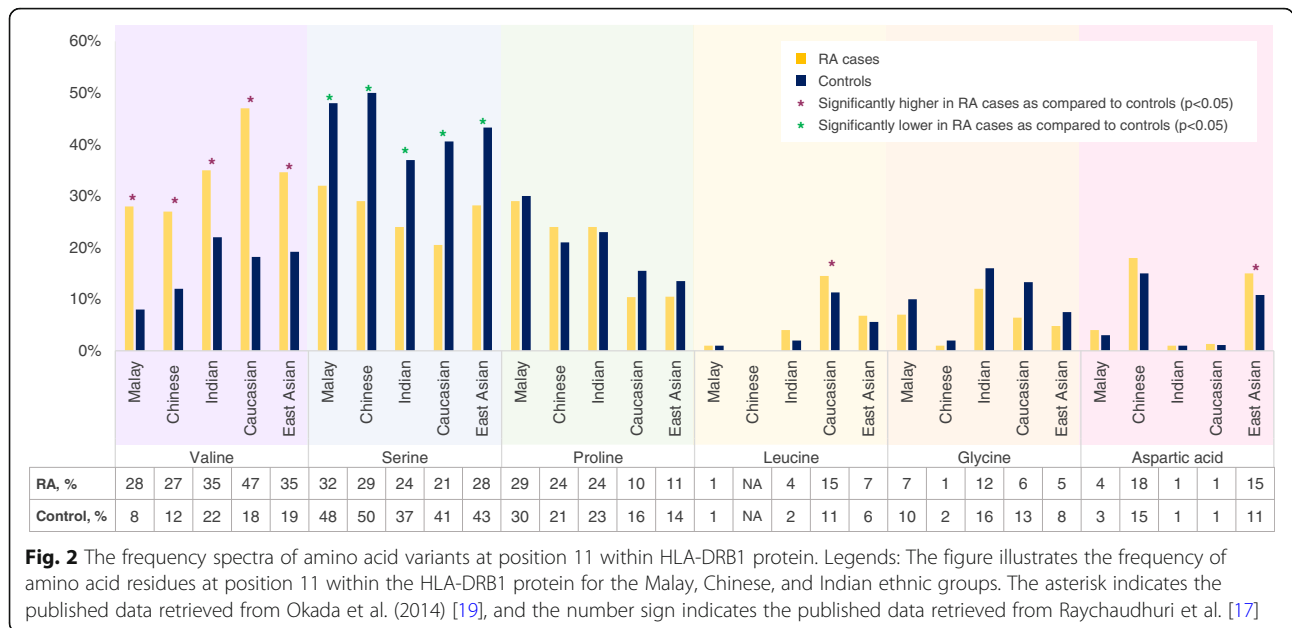


Fig. 2 The frequency spectra of amino acid variants at position 11 within HLA-DRB1 protein. Legends: The figure illustrates the frequency of amino acid residues at position 11 within the HLA-DRB1 protein for the Malay, Chinese, and Indian ethnic groups. The asterisk indicates the published data retrieved from Okada et al. (2014) [19], and the number sign indicates the published data retrieved from Raychaudhuri et al. [17]

RA associated with serine and glycine residues (i.e., serine, $OR_{cumulative} 0.49, p < 0.00001, I^2 = 0\%$; glycine, $OR_{cumulative} 0.68, p < 0.002, I^2 = 0\%$) (Supplementary Figure. 2b and c).

Discussion

Our study confirmed that the HLA-DRB1 genes with their functional characteristics are the major determinants in the pathogenesis of RA, specifically in the ACPA-positive RA subset in the multi-ethnic Malaysian population, supporting the notion of shared RA risk across different populations. We found HLA-DRB1 Val11 conferred the strongest risk effect in the ACPA-positive RA in the Malay population, one of the predominant ethnic groups in Southeast Asia. Additionally, *HLA-DQB1*03:02* demonstrated a novel and independent protective effect for ACPA-positive RA in the Malay group. Interestingly, Indian RA patients carrying HLA-DRB1 His96 are protected from risk of developing ACPA-positive RA.

The observed RA risk of HLA-DRB1 Val11 in our study population is generally concordant with the published data from different large-scale genetic association studies of Caucasian, African, and East Asian populations, in terms of the amino acid position as well as magnitude of risk. It is notable that HLA-DRB1 Val11 is located within the peptide-binding groove of the HLA Class II molecules. This suggests the pathogenic role of the identified amino acid at position 11 of the HLA-DRB1 protein (i.e., HLA-DRB1 position 11), which enables peptide binding and further recognition of MHC-peptide complexes by T cells involved in providing help to B cells expressing and producing ACPA IgG. Future study of this replicated

and validated risk variant, i.e., HLA-DRB1 position 11, is needed to generate new insights and better understanding of the implication of the risk variant for the pathophysiology of ACPA-positive RA.

The valine or leucine residue at position 11 within the HLA-DRB1 protein (i.e., HLA-DRB1 Val11 and HLA-DRB1 Leu11) is associated, predominately in the Caucasian and Spanish populations, with increased risk of severe radiographic progression in ACPA-positive RA, independent of *HLA-DRB1 SE* status [38]. The present extension of this observation to other populations, including the Malaysian population, may lead to better understanding of the pathogenic role of HLA-DRB1 Val11 and/or HLA-DRB1 Leu11 and their effect on the clinical phenotype of the disease. In this current study, the clinical data from the recruited RA cases were limited. Future studies of the implications of the identified RA risk factor on the disease progression will provide new insights/knowledge that may aid in the characterization of the RA phenotype in the clinical setting.

HLA-DRB1 His13 was reported to have the strongest association with risk of ACPA-positive RA in a mixed East Asian population comprising South Korean and Han Chinese in Beijing [19], while an earlier study in a homogenous Korean population demonstrated HLA-DRB1 Val11 was strongly associated with risk for ACPA-positive RA [17]. However, both HLA-DRB1 Val11 and HLA-DRB1 His13 are in tight LD. The HLA-DRB1 His13 observed in the mixed population study could be due to the influence of the different genetic profile of Han Chinese individuals (16.8% in RA cases and 20.2% in controls). Although the South Korean and

Chinese populations have common ancestry, the genetic profiles of these populations are distinctive [39].

Interestingly, our findings demonstrated *HLA-DQB1*03:02* allele as a novel potentially protective factor regarding risk of developing ACPA-positive RA in the Malay ethnic group. Of a different note, the *HLA-DQB1*03:02* allele was reported to associate with increased risk of developing celiac disease in the Iranian population [40]. Taken together, it is suggested that *HLA-DQB1*03:02* allele may have opposing effects, being a protective allele in one disease and a risk factor in another disease.

Recently, aspartic acid residue at position 160 within the HLA-DQA1 protein was reported to be the most significant risk factor for ACPA-positive RA in the Han Chinese population of Beijing, with HLA-DRB1 Val11 as the second strongest risk factor [22]. This pattern was however not observed in the Malaysian Chinese ethnic group in our study. The most plausible explanation is the genetic differences between the Beijing Han Chinese and the Malaysian Chinese. The Malaysian Chinese are mainly descendants of nineteenth and early twentieth century Han Chinese immigrants from Southern China (particularly the provinces of Fujian, Guandong, and Hainan) [35]. Furthermore, genetic population studies have shown that the Southern and Northern Han Chinese are two distinctive populations [39, 41].

High imputation accuracy observed in our studied dataset suggested the suitability of the Caucasian-based ImmunoChip microarray [23] and usefulness of the admixture Pan-Asian reference panel [19] for HLA imputation in the multi-ethnic Malaysian population. Based on these local evidences, utilizing the ImmunoChip microarray and admixture Pan-Asian reference panel for fine-mapping of HLA variants in other autoimmune diseases such as systemic lupus erythematosus, multiple sclerosis, and ankylosing spondylitis can be recommended.

Conclusions

Our new findings in Southeast Asian populations are in concordance with the data from other populations, suggesting HLA-DRB1 Val11 valine as the most important genetic component for the risk of ACPA-positive RA. Notably, our data also showed a novel protective allele in the *HLA-DQB1* gene (i.e., *HLA-DQB1*03:02*) associated with the risk of developing ACPA-positive RA in the Malay ethnic group. The different risk and protective residues of HLA-DRB1 amino acid at positions 11 and 96 in the Malay and Indian patients with ACPA-positive suggested different amino acid residues within the same HLA protein may promote or counteract the pathogenesis of RA. In common with the Caucasian population, there is little risk from HLA locus for ACPA-negative RA in the multi-ethnic Malaysian population.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s13075-021-02431-z>.

Additional file 1: Supplementary Table 1. Overall concordance rate of *HLA-A*, *HLA-B*, *HLA-C*, *HLA-DRB1* and *HLA-DQB1* genes in the control group, stratified by ethnicity. **Supplementary Table 2.** Classical 2-digit HLA alleles imputation accuracy in the control group, stratified by ethnicity. **Supplementary Table 3.** Classical 4-digit HLA alleles imputation accuracy in the control group, stratified by ethnicity. **Supplementary Table 4.** Number of imputed classical HLA alleles and amino acid polymorphisms achieved published genome-wide threshold of $p < 5 \times 10^{-08}$. **Supplementary Table 5.** Logistic regression results of the association between imputed HLA amino acids and alleles, and risk of developing ACPA-positive rheumatoid arthritis in the Malay, Chinese and Indian ethnic groups. **Supplementary Table 6.** Stepwise logistic regression analysis for risk of ACPA-positive RA in the Malay ethnic group. **Supplementary Figure 1.** Plot of stepwise logistic regression analysis to fine-map HLA variants as risk factor for ACPA-positive RA in the Chinese and Indian ethnic groups. **Supplementary Figure 2.** Meta-analysis of polymorphic HLA-DRB1 amino acid residues position 11 and risk of developing ACPA-positive RA in Malay, Chinese and Indian ethnic groups.

Abbreviations

HLA: Human leukocyte antigen; RA: Rheumatoid arthritis; HLA-A: Human leukocyte antigen A; HLA-B: Human leukocyte antigen B; HLA-DPB1: Human leukocyte antigen DP beta 1; HLA-DQA1: Human leukocyte antigen DQ alpha 1; ACPA: Anti-citrullinated protein antibody; MyEIRA: Malaysian Epidemiological Investigation of Rheumatoid Arthritis; OR: Odds ratio; 95% CI: 95% confidence interval; P_{GWAS} : Genome-wide significance threshold of less than 5×10^{-08} ; *HLA-DRB1* SE: Human leukocyte antigen DR beta 1 shared epitope; SNP: Single nucleotide polymorphisms; HLA-DRB1 Val11: HLA-DRB1 amino acid at position 11 with valine residue; HLA-DRB1 Leu11: HLA-DRB1 amino acid at position 11 with leucine residue; HLA-A Asn77: HLA-A amino acid at position 77 with asparagine residue; HLA-B Asp9: HLA-B amino acid at position 9 with aspartic acid; HLA-DPB1 Phe9: HLA-DPB1 amino acid at position 9 with phenylalanine residue; HLA-DQA1 Asp160: HLA-DQA1 amino acid at position 160 with aspartic acid; MHC: Major histocompatibility complex; ACR: American College of Rheumatology; anti-CCP2: Anti-cyclic citrullinated peptide second-generation (anti-CCP2); PCR-SSO: Polymerase chain reaction sequence-specific oligonucleotide; QC: Quality control; MAF: Minor allele frequency; HWE: Hardy-Weinberg equilibrium; AIC: Akaike information criterion; BIC: Bayesian information criterion; HLA-DRB1 Asn120: HLA-DRB1 amino acid at position 120 with asparagine residue; LD: Linkage disequilibrium; HLA-DRB1 His96: HLA-DRB1 amino acid at position 96 with histidine residue; Pro: Proline; Arg: Arginine; Ala: Alanine; Val: Valine; His: Histidine; Phe: Phenylalanine; Ser: Serine; Glx: Glutamic acid/ Glutamine; Gly: Glycine; Tyr: Tyrosine; Gln: Glutamine; Leu: Leucine; Lys: Lysine

Acknowledgements

The authors would like to thank the Director General of Health, Ministry of Health Malaysia, for supporting the work described in this article. Special thanks to the members of the MyEIRA study group and the rheumatologists for their dedication and excellent assistance in this study. We truly value the patients and controls for their generous participation. Dr. Daniel Ramskold's help with the data analysis using Python language is highly appreciated. We would also like to thank Ms. Janet Ahlberg for language editing.

Authors' contributions

TLK, TCL, and LP had full access to all data in this study and take responsibility for the integrity of the data and the accuracy of the data analysis. TLK, TCL, LP and LMDG were responsible for the statistical analysis, interpreting the results, and drafting the paper. TCL, LA, LK, SM, and LP conceived the study and participated in the design of the study and manuscript editing. TCL performed molecular genetics assays. TLK, TCL, LP and LMDG take responsibility for the acquisition of the experimental data, analysis, and interpretation of data. WS, LIS, HH, NSS, GSC, EM, MSMS, AMM, AR, and MO contributed through the assessment of clinical aspects for the recruited RA cases. TCL and SM are the project leaders of the MyEIRA study.

All authors were involved in revising the paper critically for important intellectual content, and all authors approved the final version of the manuscript to be published.

Funding

This study was supported by grants from the Ministry of Health, Malaysia (i.e., MRG-2005-12, JPP-IMR 07-017, JPP-IMR 07-046, JPP-IMR 08-006, JPP-IMR 08-012) and The Swedish National Research Council (DNR 348-2009-6468).

Availability of data and materials

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Ethics approval and consent to participate

This study was approved by the Medical Research and Ethics Committee, Ministry of Health Malaysia (KKM/JEPP/02 Jld 1 (86); (14) dlm. KKM/NIHSEC/08/0804/MRG-2005-12) and Stockholm Regional Ethics Committee, Sweden (2012/1381-31/1). All participants gave written informed consent.

Competing interests

The authors declare that they have no competing interests.

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Received: 4 November 2020 Accepted: 19 January 2021

Published online: 30 January 2021

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