厚生労働科学研究費補助金

肝炎等克服実用化研究事業

（肝炎等克服緊急対策研究事業）

B型肝炎の慢性化・ウイルス排除に関連する遺伝要因について、HLAアリルおよび免疫関連遺伝子群を網羅的に探索する研究

（H25-肝炎-若手-012）

平成26年度　総括研究報告書

研究代表者　澤井　裕美

平成27（2015）年3月

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研究代表者　澤井　裕美

平成27（2015）年3月

目　　次

Ⅰ．総括研究報告書

厚生労働科学研究費補助金（肝炎等克服実用化研究事業）
統括研究報告書（平成 26 年度）

B型肝炎の慢性化・ウイルス排除に関連する遺伝要因について、HLAアリルおよび免疫関連遺伝子群を網羅的に探索する研究

研究代表者：澤井　裕美　東京大学大学院医学系研究科　人類遺伝学分野　特任助教
研究協力者：馬場　菜津美、山田　佳代子

研究要旨：本研究では、B型肝炎の慢性化、ウイルス排除および病態進展に関与する遺伝要因を網羅的に探索すると共に、既知の遺伝要因である*HLA-DP*の詳細な検討を実施する事を目的とする。22か所の研究協力施設により収集された日本人検体約1,300検体に対して、*HLA-DPA1*および*HLA-DPB1*のHLAタイピングを実施した。比較の為、韓国集団、香港集団、タイ集団合計約1,800検体についてもHLAタイピングを実施した。また、新規遺伝要因同定を目的としたGWASを実施した。

HBV持続感染に関連する*HLA-DPB1*アリル同定の為の関連解析を行ったところ、先行研究と同様の2つのアリルでの関連が見られた。またそれ以外にこれまでに報告されていない2つの抵抗性アリルと1つの感受性アリルで新たに関連が示された。抵抗性/感受性アリルをヘテロで有する方がホモの場合より強い関連を示すことも明らかにした。韓国集団では日本集団と共通の2つのアリルが感受性・抵抗性に関連する事が示され、香港集団では共通のアリル1つが抵抗性に関連する事が示された。タイ集団では、日本集団とは異なるアリルの関連が示された。HBVウイルス排除に関連する*HLA-DPB1*アリル同定の為の関連解析を行い、HBV持続感染と共通のアリルでの関連を確認した。また、病態進展に関連する*HLA-DPB1*アリル同定の為の関連解析を行い、*HLA-DPB1*02:01*が慢性化のみならず病態進展にも関連するアリルとして同定された。B型肝炎の慢性化および病態進展に関連する新規の遺伝要因探索を目的としたGWASを実施し、癌化に関連する遺伝要因を同定した。

アジアにおいて、慢性化および病態進展に関連するアリルの共通性と異質性を調べる事で、病態におけるHLA-DPの機能の理解に繋がると共に、日本およびアジアにおける治療方針決定に役立つ事が期待される。また新たに同定された宿主遺伝要因とウイルス遺伝要因を組み合わせる事で、新規診断法や治療法の開発に役立てる事が期待される。

A. 研究目的

B 型肝炎ウイルス(HBV)感染後の経過は多岐に渡り、影響を及ぼす因子としては、年齢、性別、他の肝炎ウイルスとの共感染、HBV 遺伝子型等が挙げられる。近年、宿主遺伝要因についても候補遺伝子アプローチだけでなく位置的アプローチによる解析が進み、日本人を含むアジア人検体を用いたゲノムワイド関連解析(GWAS)により、HBV 持続感染やウイルス排除に *HLA-DPA1, -DPB1* の関連が示され、*HLA-DQ* の関与も示唆された。しかし GWAS ではそれ以外の強い遺伝要因は見つかっていない。

本研究では、B 型肝炎の慢性化・ウイルス排除や病態進展に関与する遺伝要因を免疫関連遺伝子を中心として網羅的に探索すると共に、既知の遺伝要因である *HLA-DP* の詳細な検討を実施する。

B. 研究方法

日本全国の研究協力施設から、サンプル（DNA 及び血清）を効率的に収集し、詳細

な臨床情報と共に管理するシステムを用いた検体取集・臨床情報の蓄積を行った。新規に収集したサンプルは受託会社にて DNA および血清を抽出・分離した後に国立国際医療研究センターへ送られた。既に DNA および血清を分離済のサンプルについては、各施設から直接国立国際医療研究センターに送られ、各施設で収集された臨床情報は、連結可能匿名化された後に国立国際医療研究センターに送られた。収集された臨床情報を元に病態毎に検体を分類し、ゲノム解析用の新たな ID が付加された。二重匿名化されたゲノム DNA と臨床情報は、東京大学大学院医学系研究科人類遺伝学分野に送られた。

新規の宿主遺伝要因を探索する事を目的として、収集した HBV 関連患者群 1,356 検体を対象としてゲノムワイド SNP タイピングを実施した。タイピングには Affymetrix 社の AXIOM ASI 1 Array（約 60 万種の SNP を搭載）を用いた。Overall call rate の平均は 99.41%、DishQC の平均は 0.969 となった。タイピング結果に基づいて、持続感染および癌化についてゲノムワイド関連解析（GWAS）を実施した。

（倫理面への配慮）

本研究を行うにあたり、代表者である澤井の所属する東京大学医学部ヒトゲノム・遺伝子解析研究倫理審査委員会から承認を得た。また、本研究で使用した検体は徳永勝士を代表とする「B 型肝炎ウイルス感染の病態別における宿主因子等について、網羅的な遺伝子解析を用い、新規診断法及び治療法の開発を行う研究」において収集されたものであり、各々の研究分担者及び研究協力者が所属する機関においても、倫理審査委員会から承認を得たのちにサンプル収集を実施した。

C. 研究結果

GWAS により HBV 持続感染およびウイルス排除に関連が示された *HLA-DP* 遺伝子の詳細な検討を目的とし、22 か所の研究協力施設により収集された日本人 HBV 患者群 489 検体、HBV 既往感染者群 335 検体、健常者群 467 検体（計 1,291 検体）に対して、*HLA-DPA1* および *HLA-DPB1* の HLA タイピングを実施した。また、日本人での解析結果と比較する為、韓国集団計 586 検体（HBV 患者群 340 検体、HBV 既往感染群 106 検体、健常者群 140 検体）、香港集団計 661 検体（HBV 患者群 281 検体、HBV 既往感染群 190 検体、健常者群 190 検体）およびタイ集団計 629 検体（HBV 患者群 390 検体、HBV 既往感染者群 113 検体、健常者群 126 検体）についても HLA タイピングを実施した（表 1）。

表 1　HLA タイピングを実施したサンプル一覧

population	Japanese	Korean	Hongkongese	Thai
total nuber of	1,291	586	661	629
HBV patients	489	340	281	390
age ave.	57.1	44.7	57.9	52.0
(min-max)	(20-84)	(18-74)	(32-86)	(21-84)
gender (M/F/ND)	337/149/3	265/75/0	239/42/0	289/101/0
Resolved	335	106	190	113
age ave.	59.7	43.1	40.0	48.2
(min-max)	(18-87)	(12-66)	(18-60)	(39-66)
gender (M/F/ND)	173/162/0	61/45/0	113/77/0	83/30/0
Healthy controls	467	140	190	126
age ave.	39.0**	33.7	26.2	46.6
(min-max)	(23-64)	(1-59)	(16-60)	(38-79)
gender (M/F/ND)	370/97/0	67/73/0	87/103/0	73/53/0

HBV 持続感染に関連する *HLA-DP* アリルを同定する為、日本人集団の HBV 患者群と健常者群の *HLA-DPB1* アリルの関連解析を行ったところ、*HLA-DBP1*05:01*（感受性アリル）、*HLA-DPB1*04:02*（感受性アリル）で有意な関連が見られ先行研究と同様の結果が得られた。また、それ以外にこれまでに報告されていない 2 つの抵抗性アリル（*HLA-DPB1*02:01*, OR = 0.71, P = 2.1×10^{-3}; *HLA-DPB1*04:01*, OR = 0.34, P = 2.4×10^{-5}）と 1 つの感受性アリル（*HLA-DPB1*09:01*, OR = 1.94, P = 3.7×10^{-6}）で新たに関連が示された。*HLA-DPA1* アリルと *HLA-DPB1* アリルの組み合わせ（ハプロタイプ）についても関連解析を実施したが、特定のハプロタイプで関連が相加的に強まる傾向は見られず、*HLA-DPB1* の特定のアリルが HBV 持続感染に対する感受性および抵抗性に重要であることが示唆された。また、抵抗性アリルをホモで有する場合とヘテロで有する場合を比較し、ヘテロで有する方がより有意な関連を示すことを明らかにした。感受性ア

リルについても同様の検討を行い、ヘテロでより有意な関連を示した。

韓国集団、香港集団、タイ集団においても *HLA-DPB1* アリルの関連解析を実施し、韓国集団では日本集団と共通のアリル（*HLA-DPB1*05:01, -DPB1*04:02*）が感受性・抵抗性に関連する事が示され、香港集団では共通のアリル（*HLA-DPB1*02:01*）が抵抗性に関連する事が示された。タイ集団では、日本集団とは異なるアリルの関連が示された。

またHBVウイルス排除に関連する *HLA-DP* アリルを同定する為、日本人集団のHBV患者群とHBc抗体陽性群（ウイルス排除群）の *HLA-DPB1* アリルの関連解析を行ったところ、*HLA-DBP1*09:01*（感受性アリル）、*HLA-DPB1*04:02*（感受性アリル）で有意な関連が見られ、*HLA-DPB1*02:01, HLA-DPB1*04:01, HLA-DPB1*04:02* でも関連の傾向が見られ($0.1<p<0.5$)、HBV持続感染と共通のアリルでの関連が示された。

更にB型肝炎慢性肝炎患者の病態進展に関連する *HLA-DPB1* アリルを同定する為、B型慢性肝炎患者群および無症候性キャリア群計261検体と肝硬変および肝癌患者群計207検体を用いた関連解析を実施した。その結果、*HLA-DPB1*02:01* が病態進展にも関連するアリルとして同定された。

B型肝炎の慢性化に関連する新規の遺伝要因探索を目的として、B型慢性肝炎患者群および無症候性HBVキャリア群（計523検体）をケース群、健常者群（計640検体）をコントロール群としてGWASを実施した。$P<10^{-4}$ を示すSNPは529か所見られ、そのうち既報の *HLA-DP* 領域が位置するHLA領域に位置するSNPは471か所にのぼった。また、B型肝炎の癌化に関連する新規の遺伝要因探索を目的として、HBV陽性肝癌患者群（473検体）をケース群、B型慢性肝炎患者群および無症候性HBVキャリア群(計516検体)をコントロール群としてGWASを実施した。$P<10^{-5}$ を示すSNPは53か所見られ、そのうちHLA領域のSNPは49か所にのぼった。49か所の連鎖不平衡を考慮して4SNPを選択し、独立の日本人検体（ケース153検体、コントロール614検体）およびアジア集団の検体（韓国：ケース148検体、コントロール126検体;香港:ケース94検体、コントロール187検体；タイ：ケース185検体、コントロール198検体）を用いて再現性を検証した。いずれの集団においてもGWASの解析結果と同様の傾向を示し、メタ解析の結果、4SNP中3SNPでゲノムワイド有意水準に達した。

D. 考察

HBV持続感染に関連する *HLA-DPB1* アリルが集団によって異なる要因としては、各国における一般集団での *HLA-DPB1* アリル頻度が大きく異なる事によると考えられる。一般集団におけるアリルの分布を詳しく調べると共に、更に検体数を増やした解析を実施する事で、*HLA-DP* アリルと病態の関わりをより詳しく調べる事が出来る。また、各国で感受性アリル・抵抗性アリルと同定されたHLA-DP分子の共通性を探る事で、病態におけるHLA-DPの機能を知る手掛かりとなる事が期待される。

B型肝炎の癌化に関連する遺伝要因の探索では、新規の遺伝要因が見出された。今後免疫関連遺伝子群を中心とした詳細な解析が必要とされる。

E. 結論

日本を含む東アジア集団サンプル約3,200検体について大規模HLAタイピングを実施し、慢性化、ウイルス排除及び病態進展に関与する *HLA-DPB1* アリルを同定した。アジアにおいて、各病態に関連するアリルの共通性と異質性を調べる事で、病態におけるHLA-DPの機能の理解に繋がると共に、日本およびアジアにおける治療方針決定に役立つ事が期待される。また、新規遺伝要因の探索を目的として実施したGWASではB型肝炎の癌化に関連する遺伝要因を同定した。宿主遺伝要因だけでなくウイルス遺伝要因と組み合わせる事で、新規診断法や治療法の開発に役立てる事が期待

される。

F. 健康危険情報
なし

G. 研究発表
1. 論文発表

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H. 知的所得権の出願・登録状況

1. 特許取得

なし

2. 実用新案登録

なし

3. その他

なし

Ⅱ．研究成果の刊行一覧

研究成果の刊行に関する一覧表

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	出版年	ページ

雑誌

発表者氏名	論文タイトル名	発表誌名	巻・号	ページ	出版年
Nishida N*, **Sawai H***; Kashiwase K, Minami M, Sugiyama M, Seto WK, Yuen MF, Posuwan N, Poovorawan Y, Ahn SH, Han KH, Matsuura K, Tanaka Y, Kurosaki M, Asahina Y, Izumi N, Kang JH, Hige S, Ide T, Yamamoto K, Sakaida I, Murawaki Y, Itoh Y, Tamori A, Orito E, Hiasa Y, Honda M, Kaneko S, Mita E, Suzuki K, Hino K, Tanaka E, Mochida S, Watanabe M, Eguchi Y, Murata K, Korenaga M, Mawatari Y, Ohashi J, Kawashima M, Tokunaga K, Mizokami M. (* equall contribution)	New susceptibility and resistance HLA-DP alleles to HBV-related diseases identified by a trans-ethnic association study in Asia.	PLoS One	9(2):	e86449	2014
Khor SS, Yang W, Kawashima M, Kamitsuji S, Zheng X, Nishida N, Sawai H, Toyoda H, Miyagawa T, Honda M, Kamatani N, and Tokunaga K.	High-accuracy imputation for HLA class I and II genes based on high-resolution SNP data of population-specific references	Pharmacogenomics J		doi: 10.1038/tpj.2015.4	2015

Ⅲ. 研究成果の刊行物・別刷

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PLOS ONE

New Susceptibility and Resistance HLA-DP Alleles to HBV-Related Diseases Identified by a Trans-Ethnic Association Study in Asia

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Abstract

Previous studies have revealed the association between SNPs located on human leukocyte antigen (*HLA*) class II genes, including *HLA-DP* and *HLA-DQ*, and chronic hepatitis B virus (HBV) infection, mainly in Asian populations. *HLA-DP* alleles or haplotypes associated with chronic HBV infection or disease progression have not been fully identified in Asian populations. We performed trans-ethnic association analyses of *HLA-DPA1*, *HLA-DPB1* alleles and haplotypes with hepatitis B virus infection and disease progression among Asian populations comprising Japanese, Korean, Hong Kong, and Thai subjects. To assess the association between *HLA-DP* and chronic HBV infection and disease progression, we conducted high-resolution (4-digit) *HLA-DPA1* and *HLA-DPB1* genotyping in a total of 3,167 samples, including HBV patients, HBV-resolved individuals and healthy controls. Trans-ethnic association analyses among Asian populations identified a new risk allele *HLA-DPB1*09:01* ($P = 1.36 \times 10^{-6}$; OR = 1.97; 95% CI, 1.50–2.59) and a new protective allele *DPB1*02:01* ($P = 5.22 \times 10^{-6}$; OR = 0.68; 95% CI, 0.58–0.81) to chronic HBV infection, in addition to the previously reported alleles. Moreover, *DPB1*02:01* was also associated with a decreased risk of disease progression in chronic HBV patients among Asian populations ($P = 1.55 \times 10^{-7}$; OR = 0.50; 95% CI, 0.39–0.65). Trans-ethnic association analyses identified Asian-specific associations of *HLA-DP* alleles and haplotypes with HBV infection or disease progression. The present findings will serve as a base for future functional studies of HLA-DP molecules in order to understand the pathogenesis of HBV infection and the development of hepatocellular carcinoma.

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Introduction

Hepatitis B virus (HBV) infection is a major global health problem, resulting in 0.5–1.0 million deaths per year [1]. The prevalence of chronic HBV infection varies. About 75% of the chronic carriers in the world live in Southeast Asia and East Pacific [2]. Due to the introduction of vaccination programs, the prevalence of HBV infection in many countries has gradually been decreasing with consequent decreases in HBV-related hepatocellular carcinoma (HCC) [3]. Although some HBV carriers spontaneously eliminate the virus, about 10–15% of carriers develop liver cirrhosis (LC), liver failure and HCC [4]. Moreover, the progression of liver disease was revealed to be associated with the presence of several distinct mutations in HBV infections [5]. Genetic variations in *STAT4* and *HLA-DQ* genes were recently identified as host genetic factors in a large-scale genome-wide association study (GWAS) for HBV-related HCC in China [6].

With regard to the genes associated with susceptibility to chronic HBV infection, *HLA-DP* and *HLA-DQ* genes were identified by GWAS in Japanese and Thai populations in 2009 [7] and 2011 [8], respectively. In addition, our previous GWAS confirmed and identified the association of SNP markers located on *HLA-DPA1* (rs3077) and *HLA-DPB1* (rs9277535) genes with susceptibility to chronic hepatitis B (CHB) and HBV clearance in Japanese and Korean subjects[9]. The significant associations of *HLA-DP* with CHB and HBV clearance have mainly been detected in Asian populations, such as Japanese [8,9], Thai [7], Chinese [10–12], and Korean [9]. In 2012, the association between *HLA-DPA1* gene SNPs and persistent HBV infection was replicated in a Germany non-Asian population for the first time; however, this showed no association with HBV infection [13]. These results seem to be explained by the fact that allele frequencies of both rs3077 (0.155, 0.587 and 0.743 for C allele, on HapMap CEU, JPT, and YRI) and rs9277535 (0.261, 0.558 and 0.103 for G allele, on HapMap CEU, JPT, and YRI) are markedly different between populations. Moreover, the previous study showed that HBsAg seropositivity rates were higher in Thailand and China (5–12%) than in North America and Europe (0.2–0.5%) [2]. These results suggest that comparative analyses of *HLA-DP* alleles and haplotypes in Asian populations would clarify key host factors of the susceptible and protective *HLA-DP* alleles and haplotypes for CHB and HBV clearance. Here, we performed trans-ethnic analyses of *HLA-DP* alleles and haplotypes in Asian populations comprising Japanese, Korean, Hong Kong and Thai individuals. The findings from this study will serve as a base for future functional studies of HLA-DP molecules.

Results

Characteristics of studied subjects

The characteristics of a total of 3,167 samples, including Japanese, Korean, Hong Kong and Thai subjects, are shown in Table 1. Each population included three groups of HBV patients, resolved individuals and healthy controls. The clinical definitions of HBV patients and resolved individuals are summarized in Materials and Methods. Some of the Japanese and all of the Korean samples overlapped with the subjects in our previous study [9,14].

We performed genotyping for *HLA-DPA1* and *HLA-DPB1* in all 3,167 samples, and a total of 2,895 samples were successfully genotyped. The characteristics of successfully genotyped samples are shown in Table S1.

Association of *HLA-DPA1* and *HLA-DPB1* alleles in Asian populations

As for a general Asian population, including 464 Japanese, 140 Korean, 156 Hong Kong, and 122 Thai subjects, five *HLA-DPA1* alleles and twenty-four *HLA-DPB1* alleles were observed (Table S2). The frequencies of *HLA-DPA1* and *HLA-DPB1* alleles were similar between Japanese and Korean subjects. On the other hand, the number of alleles with frequencies of 1–2% was larger in Hong Kong and Thai populations, despite the small sample size. Although the frequencies of *HLA-DP* alleles varied in Asian populations, *HLA-DPB1*05:01* was the most prevalent with over 30% in all populations.

The associations of *HLA-DPA1* and *HLA-DPB1* alleles with chronic HBV infection (i.e., comparison between HBV patients and healthy controls) are shown in Table S2. To avoid false positives caused by multiple testing, the significance levels were corrected based on the numbers of *HLA-DPA1* and *HLA-DPB1*

Table 1. Number of individuals in this study.

Population	Japanese	Korean	Hong Kong	Thai
Total number of samples	1,291	586	661	629
HBV patients	489	340	281	390
IC	114	-	-	-
CH	147	175	187	198
AE	21	-	-	-
LC	38	-	-	-
HCC	169	165	94	192
Mean age (y)	57.1	44.7	57.9	52.0
(min-max)	(20–84)	(18–74)	(32–86)	(21–84)
Gender (M/F)	338/151	265/75	239/42	289/101
Resolved individuals*	335	106	190	113
HCV (−)	249	106	190	113
HCV (+)	86	-	-	-
Mean age (y)	59.7	43.1	40.0	48.2
(min-max)	(18–87)	(12–66)	(18–60)	(39–66)
Gender (M/F)	173/162	61/45	113/77	83/30
Healthy controls	467	140	190	126
Mean age (y)	39.0**	33.7	26.2	46.6
(min-max)	(23–64)	(1–59)	(16–60)	(38–79)
Gender (M/F)	370/97	67/73	87/103	73/53

Abbreviation: IC, Inactive Carrier; CH, Chronic Hepatitis; AE, Acute Exacerbation; LC, Liver Cirrhosis; HCC, Hepatocellular Carcinoma.

* Resolved individuals were HBsAg negative and HBcAb positive.

** 419 of 467 healthy controls were de-identified, without information on age.

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alleles in the focal population. Briefly, the significance level was set at 0.05/(# of observed alleles at each locus) in each population (see Materials and Methods). With regard to high-risk alleles of *HLA-DPA1*, the most prevalent allele *HLA-DPA1*02:02* was significantly associated with susceptibility to HBV infection in Japanese ($P = 3.45 \times 10^{-4}$; OR = 1.39; 95% CI, 1.16–1.68) and Korean subjects ($P = 2.66 \times 10^{-5}$; OR = 1.89; 95% CI, 1.39–2.58), whereas this association was not observed in Hong Kong or Thai subjects. The association of *HLA-DPA1*02:01* with susceptibility to HBV infection was significant only in Japanese ($P = 2.61 \times 10^{-7}$; OR = 1.88; 95% CI, 1.46–2.41). The significant association of *HLA-DPA1*01:03* with protection against HBV infection was commonly observed among four Asian populations (Table S2). The pooled OR and 95% CI were 0.51 and 0.41–0.63, respectively in a meta-analysis ($P = 3.15 \times 10^{-10}$) (Fig. S1A).

As shown in Table S2, *HLA-DPB1* shows higher degree of polymorphism than *HLA-DPA1*. The most common allele in Asian populations, *HLA-DPB1*05:01*, was significantly associated with HBV susceptibility in both Japanese and Korean subjects. Although *HLA-DPB1*05:01* showed no significant association in the Hong Kong and Thai populations, the same direction of association (i.e., HBV susceptibility) was observed. Meta-analysis of the four populations revealed a significant association between *HLA-DPB1*05:01* and susceptibility to HBV infection ($P = 1.51 \times 10^{-4}$; OR = 1.45; 95% CI, 1.19–1.75) (Fig. S1B). The frequency of *HLA-DPB1*09:01* was significantly elevated in Japanese HBV patients (15.7%) as compared with healthy controls (8.7%) ($P = 3.70 \times 10^{-6}$; OR = 1.94; 95% CI, 1.45–2.62), and this association was most significant (i.e., the smallest P value) in the Japanese population. Because of lower allele frequencies of *HLA-DPB1*09:01* or lack of statistical power in the other populations, no significant associations were observed. A common allele in Thai subjects, *HLA-DPB1*13:01*, was significantly associated with susceptibility to HBV infection ($P = 2.49 \times 10^{-4}$; OR = 2.17; 95% CI, 1.40–3.47) with the same direction of associations in Japanese and Hong Kong (OR = 1.52 and 1.40, respectively).

*HLA-DPB1*04:02* was identified as the most protective allele for HBV infection in Japanese ($P = 1.59 \times 10^{-7}$; OR = 0.37; 95% CI, 0.24–0.55) and Korean subjects ($P = 1.27 \times 10^{-7}$; OR = 0.19; 95% CI, 0.10–0.38). Both *HLA-DPB1*02:01* and *HLA-DPB1*04:01* were also significantly associated with protection in the Japanese population, and the former was significantly associated with protection in Hong Kong subjects ($P = 9.17 \times 10^{-4}$; OR = 0.49; 95% CI, 0.32–0.76). This common allele among four Asian populations, *HLA-DPB1*02:01*, showed a significant association with protection against HBV infection ($P = 5.22 \times 10^{-6}$; OR = 0.68; 95% CI, 0.58–0.81) in a meta-analysis (Fig. S1B).

The frequencies of associated *HLA-DP* alleles in a comparison of HBV patients with healthy controls (Table S2) or with HBV-resolved individuals (Table S3) were similar in all four Asian populations. In the Japanese population, the associations of susceptible and protective *HLA-DPB1* alleles to chronic HBV infection seem weaker in the comparison of HBV patients with HBV-resolved individuals than in the comparison of HBV patients with healthy controls. Moreover, the results of association analyses showed no difference in the comparison of HBV patients with HBV-resolved individuals, including or excluding HCV positive individuals (Table S3). In contrast, the association became stronger in the comparison of HBV patients with HBV-resolved individuals among the Korean subjects. The protective allele *HLA-DPB1*04:01* was also identified to have a strong association with HBV clearance in Hong Kong subjects (Table S3). Moreover, in Hong Kong subjects, the *HLA-DPB1*05:01* associated with the risk for HBV infection showed lower frequency in HBV-resolved individuals (42.9%) than in the healthy controls (48.1%), which accounts for a strong association in the comparison of HBV patients with HBV-resolved individuals ($P = 6.24 \times 10^{-3}$; OR = 1.64; 95% CI, 1.14–2.36). Although the number of samples was insufficient, *HLA-DP*100:01* showed a significant association with protection against HBV infection in the Hong Kong population ($P = 3.05 \times 10^{-6}$; OR = 0.03; 95% CI, 0.0007–0.20).

Table 2. Association of number of *DPB1*02:01* alleles (i.e., 0, 1 or 2) with disease progression in CHB patients assessed by multivariate logistic regression analysis adjusted for age and sex.

Population	P value	OR (95% CI)
Japanese	0.000177	0.47 (0.32–0.70)
Korean	0.025358	0.55 (0.33–0.93)
Hong Kong	0.040842	0.46 (0.22–0.97)
Thai	0.087782	0.58 (0.31–1.08)
All*	1.55×10^{-7}	0.50 (0.39–0.65)

*Population was adjusted using dummy variables.

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As for disease progression in CHB patients among Asian populations, a protective effect of *HLA-DPB1*02:01* on disease progression was observed in the Japanese ($P = 4.26 \times 10^{-5}$; OR = 0.45; 95% CI, 0.30–0.67) and Korean populations ($P = 8.74 \times 10^{-4}$; OR = 0.47; 95% CI, 0.29–0.75) (Table S4). Multivariate logistic regression analysis adjusted for age and sex revealed that the number of *DPB1*02:01* alleles (i.e., 0, 1, or 2) was significantly associated with disease progression in CHB patients in Japanese ($P = 1.77 \times 10^{-4}$; OR = 0.47; 95% CI, 0.32–0.70) (Table 2). Moreover, protective effects of *DPB1*02:01* on disease progression in Asian populations ($P = 1.55 \times 10^{-7}$; OR = 0.50; 95% CI, 0.39–0.65) were detected in a multivariate logistic regression analysis adjusted for age, gender, and population (Table 2).

Associations of *DPA1-DPB1* haplotypes in Asian populations

The estimated frequencies of *HLA DPA1-DPB1* haplotypes are shown in Table S5. The most frequent haplotype among the four Asian populations was *DPA1*02:02-DPB1*05:01*. The number of haplotypes with low frequencies of 1–2% was 10 in both Japanese and Korean subjects, whereas more haplotypes appeared with frequencies of 1–2% in Hong Kong and Thai subjects. The associations of *DPA1-DPB1* haplotypes with HBV infection are shown in Table S5. In the Japanese population, *DPA1*02:01-DPB1*09:01* showed the most significant association with susceptibility to HBV infection ($P = 3.38 \times 10^{-6}$; OR = 1.95; 95% CI, 1.46–2.64). The most common haplotype in the four Asian populations, *DPA1*02:02-DPB1*05:01*, was found to be significantly associated with susceptibility to HBV infection in the Japanese and Korean subjects ($P = 7.40 \times 10^{-4}$; OR = 1.37; 95% CI, 1.14–1.66 for Japanese, and $P = 4.50 \times 10^{-6}$; OR = 2.02; 95% CI, 1.48–2.78 for Korean). In the Thai subjects, *HLA-DPB1*13:01* was the most significant risk allele for HBV infection (Table S2); however, no significant associations were found for the three different haplotypes bearing *HLA-DPB1*13:01*: *DPA1*02:01-DPB1*13:01*, *DPA1*02:02-DPB1*13:01*, and *DPA1*04:01-DPB1*13:01*,indicating that the association of *HLA-DPB1*13:01* with susceptibility to HBV infection did not result from a specific *DPA1-DPB1* haplotype or combination with a specific *DPA1* allele.

In the Japanese population, both haplotypes *DPA1*01:03-DPB1*04:01* and *DPA1*01:03-DPB1*04:02* showed significant associations with protection against HBV infection ($P = 1.17 \times 10^{-5}$; OR = 0.32; 95% CI, 0.18–0.56 for *DPA1*01:03-DPB1*04:01* and $P = 1.95 \times 10^{-7}$; OR = 0.37; 95% CI, 0.24–0.55 for *DPA1*01:03-DPB1*04:02*). In the Korean subjects, a significant association of *DPA1*01:03-DPB1*04:02* was also demonstrated; however, no association was observed for *DPA1*01:03-DPB1*04:01*. Because the observed number of each haplotype was small, none of the other haplotypes showed a significant association with protection against HBV infection.

In order to identify trans-ethnic DPA1-DPB1 haplotypes associated with HBV infection, a meta-analysis was performed. A meta-analysis further revealed that the *DPA1*01:03-DPB1*02:01* haplotype was significantly associated with protection against HBV infection ($P = 1.45 \times 10^{-5}$; OR = 0.69; 95% CI, 0.58–0.82) (Fig. S1C).

Discussion

Among 2.2 billion individuals worldwide who are infected with HBV, 15% of these are chronic carriers. Of chronic carriers, 10–15% develops LC, liver failure and HCC, and the remaining individuals eventually achieve a state of nonreplicative infection, resulting in HBsAg negative and anti-HBc positive, i.e. HBV-resolved individuals. To identify host genetic factors associated with HBV-related disease progression may lead HBV patients to discriminate individuals who need treatment.

The *HLA-DPA1* and *HLA-DPB1* genes were identified as host genetic factors significantly associated with CHB infection, mainly in Asian populations [7–12], and not in European populations [13]. In the previous association analyses of *HLA-DPB1* alleles with HBV infection, one risk allele *HLA-DPB1*05:01* (OR = 1.52; 95% CI, 1.31–1.76), and two protective alleles, *HLA-DPB1*04:01* (OR = 0.53; 95% CI, 0.34–0.80) and *HLA-DPB1*04:02* (OR = 0.47; 95% CI, 0.34–.64), were identified in the Japanese population [7]. In this study, we further identified a new risk allele *HLA-DPB1*09:01* (OR = 1.94; 95% CI, 1.45–2.62) for HBV infection and a new protective allele *HLA-DPB1*02:01* (OR = 0.71; 95% CI, 0.56–0.89) in the Japanese population, in addition to the previously reported alleles (Table S2) [7]. The discrepancy in the association of *HLA-DPB1*09:01* allele with risk for HBV infection in a previous study [7] results from the elevated frequency of *HLA-DPB1*09:01* in the controls (12.2%), which is higher than our controls (8.7%). In this study, healthy subjects were recruited as controls. In contrast, individuals that were registered in BioBank Japan as subjects with diseases other than CHB were recruited as controls in the previous study [7], which may have included patients with diseases with which *HLA-DPB1*09:01* is associated. Although no significant association of *HLA-DPB1*09:01* with risk for HBV infection was observed in the Korean subjects, *HLA-DPB1*09:01* appears to have a susceptible effect on HBV infection, as it showed the same direction of association. When the association analyses in Japanese and Korean subjects were combined in meta-analysis, the association was statistically significant ($P = 1.36 \times 10^{-6}$; OR = 1.97; 95% CI, 1.50–2.59). Thus, *HLA-DPB1*09:01* may be a Northeast Asian-specific allele associated with risk for HBV infection.

Moreover, a significant association of *HLA-DPB1*13:01* with risk of HBV infection (OR = 2.17; 95% CI, 1.40–3.47) was identified in the Thai subjects. However, the frequency of *HLA-DPB1*13:01* in Thai healthy controls (11.5% in the present study) reportedly varies, ranging from 15.4% to 29.5%, due to the population diversity [15–17]. Therefore, a replication analysis is required to confirm the association of *HLA-DPB1*13:01* with HBV infection in the Thai subjects. There were four other marginally associated *HLA-DPB1* alleles with low allele frequencies below 5% in HBV patients and healthy controls, including *HLA-DPB1*28:01*, *-DPB1*31:01*, *-DPB1*100:01*, and *-DPB1*105:01*, in the Hong Kong and Thai subjects. Because these infrequent alleles may have resulted from false positive associations, the association needs to be validated in a large number of subjects.

*HLA-DPB1*02:01* showed a significant association with protection against HBV infection in both Japanese and Hong Kong populations (Table S2); however, the *HLA-DPB1*02:01* allele was not associated with HBV infection in the previous study [7]. Although *HLA-DPB1*02:01* showed no association in either Korean or Thai populations, a significant association of *HLA-DPB1*02:01* with protection against HBV infection among four Asian populations was detected in meta-analysis ($P = 5.22 \times 10^{-6}$; OR = 0.68; 95% CI, 0.58–0.81) (Fig. S1B). We therefore conclude that the present finding is not a false positive.

A recent report showed that *HLA-DPB1*02:01:02*, **02:02*, **03:01:01*, **04:01:01*, **05:01*, **09:01*, and **14:01* were significantly associated with response to booster HB vaccination in Taiwan neonatally vaccinated adolescents [18]. The *HLA-DPB1*02:01:02*, **02:02*, **03:01:01*, **04:01:01*, and **14:01* were significantly more frequent in recipients whose post-booster titers of antibodies against HBV surface antigen (anti-HBs) were detectable, on the other hand, *HLA-DPB1*05:01* and **09:01* were significantly more frequent in recipients who were undetectable. Moreover, the *HLA-DPB1*05:01* and **09:01* significantly increase the likelihoods of undetectable pre-booster anti-HBs titers. These results seem consistent with our findings, in which *HLA-DPB1*05:01* and **09:01* are associated with susceptibility to chronic hepatitis B infection.

We also identified a protective effect of *HLA-DPB1*02:01* allele on disease progression in Asian populations. Previous studies identified the association of HLA class II genes including *HLA-DQ* and *HLA-DR* with development of HBV related hepatocellular carcinoma in the Chinese population [6,19,20]. In this study using Japanese and Korean samples, we identified significant associations between *HLA-DPB1*02:01* and disease progression in CHB patients ($P = 4.26 \times 10^{-5}$; OR = 0.45; 95% CI, 0.30–0.67, for Japanese and $P = 8.74 \times 10^{-4}$; OR = 0.47; 95% CI, 0.29–0.75 for Korean) (Table S4). Although the association of *HLA-DPB1*02:01* with disease progression was weaker after adjustment for age and gender in Korean subjects ($P = 2.54 \times 10^{-2}$; OR = 0.55; 95% CI, 0.33–0.93), the same direction of association was observed (i.e. protective effect on disease progression) (Table 2). The protective effects of *HLA-DPB1*02:01* on disease progression showed a significant association after adjustment for age and gender in the Japanese population ($P = 1.77 \times 10^{-4}$; OR = 0.47; 95% CI, 0.32–0.70); moreover, a significant association between *HLA-DPB1*02:01* was observed among four Asian populations, under which population was adjusted by using dummy variables in a multivariate logistic regression analysis ($P = 1.55 \times 10^{-7}$; OR = 0.50; 95% CI, 0.39–0.65) (Table 2).

The *HLA-DPA1* and *HLA-DPB1* belong to the HLA class II alpha and beta chain paralogues, which make a heterodimer consisting of an alpha and a beta chain on the surface of antigen presenting cells. This HLA class II molecule plays a central role in the immune system by presenting peptides derived from extracellular proteins. We identified two susceptible haplotypes (*DPA1*02:02-DPB1*05:01* and *DPA1*02:01-DPB1*09:01*) and three protective haplotypes (*DPA1*01:03-DPB1*04:01*, *DPA1*01:03-DPB1*04:02*, and *HLA-DPA1*01:03-DPB1*02:01*) to chronic hepatitis B infection, which may result in different binding

affinities between HLA-DP subtypes and extracellular antigens. Although functional analyses of HLA-DP subtypes to identify HBV-related peptides are not fully completed, identification of susceptible and protective haplotypes as host genetic factors would lead us to understand the pathogenesis of HBV infection including viral factors.

In summary, we identified a new risk allele *HLA-DPB1*09:01*, which was specifically observed in Northeast Asian populations, Japanese and Korean. Moreover, a new protective allele *HLA-DPB1*02:01* was identified among four Asian populations: Japanese, Korean, Hong Kong and Thai. The protective allele *HLA-DPB1*02:01* was associated with both chronic HBV infection and disease progression in chronic HBV patients. Identification of a total of five alleles, including two risk alleles (*DPB1*09:01* and *DPB1*05:01*) and three protective alleles (*DPB1*04:01*, *DPB1*04:02* and *DPB1*02:01*), would enable HBV-infected individuals to be classified into groups according to the treatment requirements. Moreover, the risk and protective alleles for HBV infection and disease progression, identified in this study by means of trans-ethnic association analyses, would be key host factors to recognize HBV-derived antigen peptides. The present results may lead to subsequent functional studies into HLA-DP molecules and viral factors in order to understand the pathogenesis of HBV infection and development of hepatocellular carcinoma.

Materials and Methods

Ethics Statement

All study protocols conform to the relevant ethical guidelines, as reflected in the *a priori* approval by the ethics committee of National Center for Global Health and Medicine, and by the ethics committees of all participating universities and hospitals, including The University of Tokyo, Japanese Red Cross Kanto-Koshinetsu Block Blood Center, The University of Hong Kong, Chulalongkorn University, Yonsei University College of Medicine, Nagoya City University Graduate School of Medical Sciences, Musashino Red Cross Hospital, Tokyo Medical and Dental University, Teine Keijinkai Hospital, Hokkaido University Graduate School of Medicine, Kurume University School of Medicine, Okayama University Graduate School of Medicine, Yamaguchi University Graduate School of Medicine, Tottori University, Kyoto Prefectural University of Medicine, Osaka City University Graduate School of Medicine, Nagoya Daini Red Cross Hospital, Ehime University Graduate School of Medicine, Kanazawa University Graduate School of Medicine, National Hospital Organization Osaka National Hospital, Iwate Medical University, Kawasaki Medical College, Shinshu University School of Medicine, Saitama Medical University, Kitasato University School of Medicine, Saga Medical School, and University of Tsukuba.

Written informed consent was obtained from each patient who participated in this study and all samples were anonymized. For Japanese healthy controls, 419 individuals were de-identified with information about gender, and all were recruited after obtaining verbal informed consent in Tokyo prior to 1990. For the 419 Japanese healthy individuals, written informed consent was not obtained because the blood sampling was conducted before the "Ethical Guidelines for Human Genome and Genetic Sequencing Research" were established in Japan. Under the condition that DNA sample is permanently de-linked from the individual, this study was approved by the Research Ethics Committee of National Center for Global Health and Medicine.

Characteristics of studied subjects

All of the 3,167 genomic DNA samples were collected from individuals with HBV, HBV-resolved individuals (HBsAg-negative and anti-HBc-positive) and healthy controls at 26 multi-center hospitals throughout Japan, Korea, Hong Kong, and Thailand (Table 1). In a total of 1,291 Japanese and 586 Korean samples, 1,191 Japanese individuals and all 586 Korean individuals were included in our previous study [9]. With regard to additional Japanese individuals, we collected samples from 48 healthy controls at Kohnodai Hospital, and 52 HBV patients at Okayama University Hospital and Ehime University Hospital, including 26 individuals with LC and 26 individuals with HCC. A total of 661 Hong Kong samples and 629 Thai samples were collected at Queen Mary Hospital and Chulalongkorn University, respectively.

HBV status was measured based on serological results for HBsAg and anti-HBc with a fully automated chemiluminescent enzyme immunoassay system (Abbott ARCHITECT; Abbott Japan, Tokyo, Japan, or LUMIPULSE f or G1200; Fujirebio, Inc., Tokyo, Japan). For clinical staging, inactive carrier (IC) state was defined by the presence of HBsAg with normal ALT levels over 1 year (examined at least four times at 3-month intervals) and without evidence of liver cirrhosis. Chronic hepatitis (CH) was defined by elevated ALT levels (>1.5 times the upper limit of normal [35 IU/L]) persisting over 6 months (by at least 3 bimonthly tests). Acute exacerbation (AE) of chronic hepatitis B was defined as an elevation of ALT to more than 10 times the upper limit of normal (ULN, 58 IU/L) and bilirubin to at least three times ULN (15 μmol/L). LC was diagnosed principally by ultrasonography (coarse liver architecture, nodular liver surface, blunt liver edges and hypersplenism), platelet counts<100,000/cm^3, or a combination thereof. Histological confirmation by fine-needle biopsy of the liver was performed as required. HCC was diagnosed by ultrasonography, computerized tomography, magnetic resonance imaging, angiography, tumor biopsy or a combination thereof.

The Japanese control samples from HBV-resolved subjects (HBsAg-negative and anti-HBc-positive) at Nagoya City University-affiliated healthcare center were used by comprehensive agreement (anonymization in a de-identified manner) in this study. Some of the unrelated and anonymized Japanese healthy controls were purchased from the Japan Health Science Research Resources Bank (Osaka, Japan). One microgram of purified genomic DNA was dissolved in 100 μl of TE buffer (pH 8.0) (Wako, Osaka, Japan), followed by storage at −20°C until use.

Genotyping of *HLA-DPA1* and *HLA-DPB1* alleles

High resolution (4-digit) genotyping of *HLA-DPA1* and *-DPB1* alleles was performed for HBV patients, resolved individuals, and healthy controls in Japan, Korea, Hong Kong, and Thailand. LABType SSO HLA DPA1/DPB1 kit (One Lambda, CA) and a Luminex Multi-Analyte Profiling system (xMAP; Luminex, Austin, TX) were used for genotyping, in according with the manufacturer's protocol. Because of the small quantity of genomic DNA in some Korean samples, we performed whole genome amplification for a total of 486 samples using GenomiPhi v2 DNA Amplification kit (GE Healthcare Life Sciences, UK), in accordance with the manufacturer's instruction.

A total of 2,895 samples were successfully genotyped and characteristics of these samples are summarized in Table S1.

Statistical analysis

Fisher's exact test in two-by-two cross tables was used to examine the associations between *HLA-DP* allele and chronic HBV infection or disease progression in chronic HBV patients,

using statistical software R2.9. To avoid false-positive results due to multiple testing, significance levels were adjusted based on the number of observed alleles at each locus in each population. For *HLA-DPA1* alleles, the number of observed alleles was 3 in Japanese, 4 in Korean, 5 in Hong Kong, and 5 in Thai subjects. Therefore, the significant levels for α were set at $\alpha = 0.05/3$ in Japanese, $\alpha = 0.05/4$ in Korean, $\alpha = 0.05/5$ in Hong Kong, and $\alpha = 0.05/5$ in Thai subjects. In the same way, significant levels for *HLA-DPB1* alleles were $\alpha = 0.05/10$, $0.05/11$, $0.05/12$, and $0.05/16$, respectively. Multivariate logistic regression analysis adjusted for age and sex (used as independent variables) was applied to assess associations between the number of *DPB1*02:01* alleles (i.e., 0, 1, or 2) and disease progression in CHB patients. To examine the effect of *DPB1*02:01* allele on disease progression in all populations, population was further adjusted by using three dummy variables (i.e., (c1, c2, c3) = (0, 0, 0) for Japanese, (1, 0, 0) for Korean, (0, 1, 0) for Hong Kong, and (0, 0, 1) for Thai) in a multivariate logistic regression analysis. We obtained the following regression equation: logit(p) = −3.905+0.083*age+(−0.929)*sex+(−0.684)**DPB1*02:01*+1.814*c1+(−0.478)*c2+0.782*c3. Significance levels in the analysis of disease progression in CHB patients were set as $\alpha = 0.05/10$ in Japanese, $\alpha = 0.05/11$ in Korean, $\alpha = 0.05/15$ in Hong Kong, and $\alpha = 0.05/15$ in Thai subjects. The phase of each individual (i.e., a combination of two *DPA1-DPB1* haplotypes) was estimated using PHASE software [21], assuming samples are selected randomly from a general population. In comparison of the estimated *DPA1-DPB1* haplotype frequencies, significant levels were set as $\alpha = 0.05/14$ in Japanese, $\alpha = 0.05/17$ in Korean, $\alpha = 0.05/17$ in Hong Kong, and $\alpha = 0.05/18$ in Thai subjects. Meta-analysis was performed using the DerSimonian-Laird method (random-effects model) in order to calculate pooled OR and its 95% confidence interval (95% CI). We applied meta-analysis for alleles with frequency>1% in all four Asian populations. The significance levels in meta-analysis were adjusted by the total number of statistical tests; $\alpha = 0.05/20$ for *DPA1* alleles, $\alpha = 0.05/57$ for *DPB1* alleles, and $\alpha = 0.05/74$ for *DPA1-DPB1* haplotypes.

Supporting Information

Figure S1 Comparison of odds ratios in association analyses for *HLA-DP* with chronic HBV infection among four Asian populations: (A) *HLA-DPA1* alleles; (B) *HLA-DPB1* alleles; and (C) *HLA DPA1-DPB1* haplotypes. Meta-analysis was performed using the DerSimonian-Laird method (random-effects model) to calculate pooled OR and its 95% confidence interval (95% CI). Bold depicts a statistically significant association after correction of significance level.
(DOCX)

Table S1 Individuals with successfully genotyped for *HLA-DPA1* and *HLA-DPB1*.
(DOCX)

Table S2 Frequencies of HLA-DP alleles in HBV patients and healthy controls among Asian populations.
(XLSX)

Table S3 Frequencies of HLA-DP alleles in HBV patients and resolved individuals among Asian populations.
(XLSX)

Table S4 Associations of HLA-DPB1 alleles with disease progression in CHB patients among Asian populations.
(XLSX)

Table S5 Estimated frequencies of HLA DPA1-DPB1 haplotypes in HBV patients and healthy controls among Asian populations.
(XLSX)

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Author Contributions

Conceived and designed the experiments: NN HS MS KT M. Mizokami. Performed the experiments: NN HS KK Y. Mawatari M. Kawashima M. Minami. Analyzed the data: NN HS M. Kawashima JO. Contributed reagents/materials/analysis tools: W-KS M-FY NP YP SHA K-HH K. Matsuura YT M. Kurosaki YA NI J-HK SH TI KY IS Y. Murawaki YI AT EO YH MH SK EM KS KH ET SM MW YE NM K. Murata M. Korenaga KT M. Mizokami. Wrote the paper: NN HS JO KT M. Mizokami.

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ORIGINAL ARTICLE

High-accuracy imputation for *HLA* class I and II genes based on high-resolution SNP data of population-specific references

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Statistical imputation of classical human leukocyte antigen (*HLA*) alleles is becoming an indispensable tool for fine-mappings of disease association signals from case–control genome-wide association studies. However, most currently available *HLA* imputation tools are based on European reference populations and are not suitable for direct application to non-European populations. Among the *HLA* imputation tools, The HIBAG R package is a flexible *HLA* imputation tool that is equipped with a wide range of population-based classifiers; moreover, HIBAG R enables individual researchers to build custom classifiers. Here, two data sets, each comprising data from healthy Japanese individuals of difference sample sizes, were used to build custom classifiers. *HLA* imputation accuracy in five *HLA* classes (*HLA-A*, *HLA-B*, *HLA-DRB1*, *HLA-DQB1* and *HLA-DPB1*) increased from the 82.5–98.8% obtained with the original HIBAG references to 95.2–99.5% with our custom classifiers. A call threshold (CT) of 0.4 is recommended for our Japanese classifiers; in contrast, HIBAG references recommend a CT of 0.5. Finally, our classifiers could be used to identify the risk haplotypes for Japanese narcolepsy with cataplexy, *HLA-DRB1*15:01* and *HLA-DQB1*06:02*, with 100% and 99.7% accuracy, respectively; therefore, these classifiers can be used to supplement the current lack of *HLA* genotyping data in widely available genome-wide association study data sets.

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INTRODUCTION

Human Leukocyte Antigen (HLA) represents the primary system, which encodes cell-surface protein that presents the specific antigen peptides for the host immune system. Specific HLA proteins have been implicated as the major susceptible factors for adverse drug reactions, transplant/graft rejection and a wide range of autoimmune and infectious diseases. The *HLA*, or major histocompatibility complex (*MHC*), region in humans is located on chromosome 6p21.3 *HLA* loci, and HLA proteins are highly polymorphic because of natural selection against a wide range of pathogens.[1] For example, as of October 2014, the IMGT/HLA database contained up to 12 000 *HLA* alleles,[2] and *HLA-B* and *HLA-DRB* were the most polymorphic in *HLA* class I and *HLA* class II genes, with 3693 and 1684 two-field alleles, respectively.

Over the years, different methodologies have been developed for genotyping *HLA* alleles, from classical two-digit serotyping to four-or-more-digit DNA-based typing methods. However, *HLA* genotyping is still notorious for being time consuming and costly for research studies that involve thousands of samples. To overcome these problems, methods for predicting *HLA* genotypes based on single nucleotide polymorphisms (SNPs) have been developed.[3,4] However, the utility of such prediction methods is limited to specific populations for which a particular prediction system is built. An alternative method uses multiple SNPs in the proximity of *HLA* regions to predict *HLA* genotypes. Leslie *et al.*[5] developed an *HLA* prediction system based on identity-by-descents model; this system uses multiple SNPs to infer haplotype information. Using the same statistical algorithm Dilthey *et al.*[6] developed an integrative software program, HLA*IMP, based on SNP data from European populations with a modification of the SNP selection process, which increased the imputation accuracy. A subsequently developed software program, HLA*IMP:02,[7] based on SNP data from multiple populations that can accommodate haplotypic heterogeneity, is also available. Each version of HLA*IMP required users to upload the genotype data to a secure, online server; this requirement may exclude certain research groups from using HLA*IMP.

SNP2HLA is an *HLA* and amino-acid imputation software program built based on the imputation algorithm used for the software package BEAGLE.[8] SNP2HLA has enabled researchers to interrogate functional coding variants within *HLA* genes that might be causal for certain diseases. Non-synonymous changes within *HLA* genes might cause variations in the binding affinity of the respective HLA protein, but the exact underlying mechanisms of how such changes contribute to disease susceptibilities remains unknown.

The HIBAG R package is an another tool for *HLA* genotype imputation based on the attribute bagging method.[9] Attribute bagging maximizes the advantages of bootstrap aggregation and the random variables selection methods to improve accuracy of *HLA* imputation.[10] In brief, ensemble classifiers are built by randomly selecting sets of individuals from a training data set and randomly selecting representative SNP markers from a set of available SNP sets. The ensemble classifiers are then used as

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