p-value for Japanese samples was 10^{-29}, indicating a definite association with type II diabetes, and the same clear association was found for Korean and Chinese samples. However, although European samples showed the same tendency of the odds ratio, the *p*-value was at a level of no more than 10^{-4}.

Table 1. Population differences of susceptibility genes to type II diabetes.

Gen (SNP)	Population	Odds Ratio	*p*	Minor Allele Frequency
TCF7L2 (rs7903146)	European [8–11]	1.37	1.0×10^{-48}	0.31/0.25
TCF7L2 (rs7903146)	Japanese [14]	1.70	7.0×10^{-4}	0.05/0.02
KCNQ1 (rs2237892)	European [12]	1.29	7.8×10^{-4}	0.03/0.05
KCNQ1 (rs2237892)	Japanese [12]	1.43	3.0×10^{-29}	0.31/0.40

In other words, the main type II diabetes-susceptibility genes for European and East Asian populations, respectively, are, in fact, shared susceptibility genes by both populations, but because they differ greatly in frequency, their contribution in each respective population is different.

Several genetic factors, in addition to environmental factors, such as stress, are involved in the onset of narcolepsy, one of the hypersomnia. In the past, the only gene well established as a genetic factor for narcolepsy was *HLA-DR/DQ* [15]; then, we carried out a GWAS to search for new genetic factors [16]. As a result, an SNP located between *CPT1B* and *CHKB* on Chromosome 22 was found to be associated with narcolepsy. Japanese and Koreans were found to have similar allele frequency and both showed a significant association. However, although the odds ratio showed similar trends in European Americans and African Americans, we could not find a significant difference association, because of the low frequency of the susceptibility allele. We have also experienced significant population differences in other diseases, including tuberculosis [17], rheumatoid arthritis [18], glaucoma [19] and primary biliary cirrhosis [20].

The above diseases serve as examples of different contributions of multiple genetic factors in each population. Consequently, the study of each individual population would be essential to build a complete picture of the important genetic factors to complex diseases in the various human populations.

2.2. Susceptibility Genes Common to Different Diseases

There has been an increase in the number of reports of genetic factors that are common to different diseases. *GPC5* (glypican-5) has been found to be a susceptibility gene common to nephrotic syndrome diseases, such as membranous nephropathy, immunoglobulin A nephropathy and diabetic nephropathy (Table 2) [21]. We further confirmed the expression of the GPC5 protein in the glomerular podocytes and showed that the risk allele is associated with a high level of GPC5 expression.

Table 2. Common susceptibility gene *GPC5* (glypican 5) for acquired nephrotic syndrome [21].

Panel	Case: Minor Allele Frequency	Control: Minor Allele Frequency	*p* *	Odds Ratio
1	0.237	0.167	5.8×10^{-3}	2.33 (1.25–4.35)
2	0.195	0.159	2.0×10^{-5}	3.44 (1.89–6.25)
3	0.224	0.174	8.7×10^{-6}	2.39 (1.61–3.55)
Combined	0.219	0.168	6.0×10^{-11}	2.54 (1.91–3.40)

* Based on the recessive model of the minor allele (GG + GA *vs.* AA).

Meta-analysis of the largest-scale GWAS in Japan on rheumatoid arthritis (RA) led to the discovery of susceptibility genes that are common to various different autoimmune disorders [18]. The GWAS was performed on approximately 4000 patients and 17,000 controls, and a replication study was carried out with 5000 patients and 22,000 controls. In addition to previously reported susceptibility genes, nine new susceptibility genes were discovered. Among these are several susceptibility genes that have been also reported for systemic lupus erythematosus (SLE) and Graves' disease.

Another example in our recent experience was primary biliary cirrhosis [20]. We performed a GWAS by a nation-wide collaboration; as a result, we discovered two new susceptibility genes. Interestingly, one of these, *TNFSF15*, has also been reported as a susceptibility gene for inflammatory bowel disease, including Crohn's disease and ulcerative colitis. There are numerous other reports of genetic factors that are found to be common to various autoimmune and inflammatory diseases [22,23].

The presence of common susceptibility genes for different diseases suggests that at least part of the pathogenic mechanism of these diseases is shared. These results may contribute to the elucidation of the pathogenic mechanism of these diseases and to the development of new therapies.

2.3. Towards the Understanding of Pathogenic Mechanisms

As mentioned earlier, the new narcolepsy-susceptibility region, *CPT1B/CHKB*, was discovered through a GWAS performed to search for genetic factors other than the established factor, *HLA* [16]. Subjects possessing the risk allele of the susceptibility SNP showed significantly lower levels of mRNA expression of both *CPT1B* and *CHKB*. We also observed that narcolepsy patients show abnormally low levels of carnitine [24], on which *CPT1B* (carnitine palmitoyltransferase 1B) is relevant, and that carnitine improves the sleep of the patients [25]. Carnitine is known as the transporter of long-chain fatty acids into mitochondria, thus playing a crucial role in energy production.

Moreover, the new susceptibility gene, *TRA* (T cell receptor α), was discovered through a GWAS performed by a joint international research group [26]. SNPs located in the J region of *TRA* showed significant associations with narcolepsy in European and Asian populations. *TRA* and *HLA* are key molecules in the regulation of immune response in the acquired immunity. The same joint international research group also found that a polymorphism of *P2RY11*, which is also involved in the regulation of the immune system, is associated with narcolepsy [27]. From these results, it may be assumed that narcolepsy onset has at least two mechanisms: both autoimmunity to orexin (hypocretin)-producing cells and a disorder of fatty acid β-oxidation.

If we appreciate that multiple susceptibility genes that have been discovered belong to specific pathways or networks, they will provide useful hints toward clarifying the mechanism of disease onset or disease formation and also developing new drugs.

3. Identified Response Genes to Drugs/Therapies

3.1. Development of New Gene Tests

GWAS studies are extremely useful in the search for drug-response genes. We performed a GWAS as part of a multi-institutional joint research group investigating hepatitis C virus related diseases. As a result of this GWAS, we discovered that *IL28B* on Chromosome 19 was strongly associated with

non-responder patients to the combined therapy of PEGylated interferon-alpha and ribavirin for chronic hepatitis C [28]. This was a completely unexpected result. The GWAS was performed on only 78 non-responders and 64 responders to this therapy; nevertheless a *p*-value at the level of 10^{-12} was obtained, reaching the genome-wide significance level (Figure 1). About 70%–80% of the non-responding patients possessed the minor alleles of several SNPs in the *IL28B* region, and combining the replication study data, the *p*-value was 10^{-27}–10^{-32} and the odds ratio was 17–30 (Figure 2).

Figure 1. A genome-wide association study (GWAS) on the response to the combined therapy of PEGylated interferon-alpha and ribavirin for chronic hepatitis C identified two SNPs on Chromosome 19 [28].

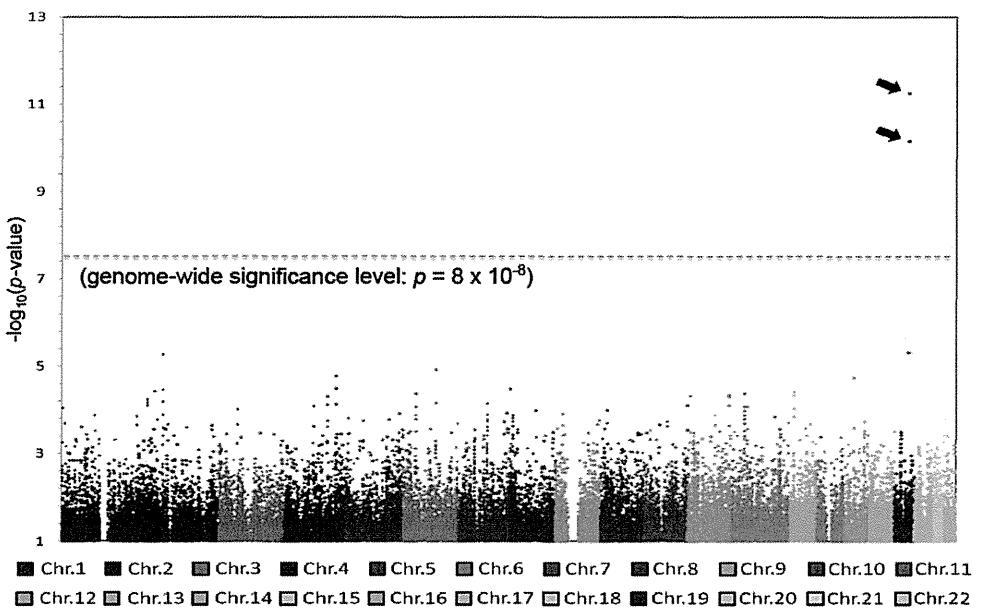
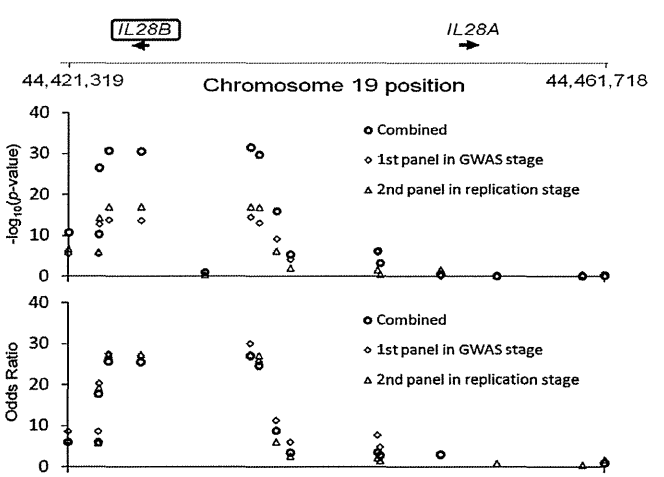


Figure 2. The strong association of *IL28B* with therapy response for chronic hepatitis C: 80% of non-responders possess the minor allele [28].



Response to the interferon-alpha therapy had been considered to be determined mainly by the virus genotype and concentration. However, the discovery that response is, in fact, mostly determined by a human genetic factor had a major impact. *IL28B* SNP typing has already been introduced into the routine clinical testing in Japan and is used as important reference data in the determination of therapeutic strategies.

3.2. Identification of New Therapeutic Targets

The discovery of *IL28B*, which is strongly associated with response to treatment for hepatitis C, indicated another highly interesting possibility. *IL28B* is a member of the interferon λ family and is assumed to exhibit its defensive activity against viral infection mediated by similar receptors and intracellular signal transduction pathway as interferon α, which was used in the treatment of hepatitis C. *IL28B* itself is therefore expected to be a powerful contender for the development of new hepatitis C drugs. In fact, *IL-29*, a member of the same family, has already been subjected to clinical trial for a new drug.

In addition to the above, genes involved in response to many drugs have been reported, and an increasing number of genetic factors are being identified for the first time as a result of GWAS. Drug-response genes generally tend to show greater odds ratios than disease-susceptibility genes, so that even with a relatively small sample size, there is a high likelihood of being able to identify the relevant gene. Ever greater results may therefore be expected in the future.

4. Particular Importance of HLA

4.1. Immune-Mediated Diseases and HLA

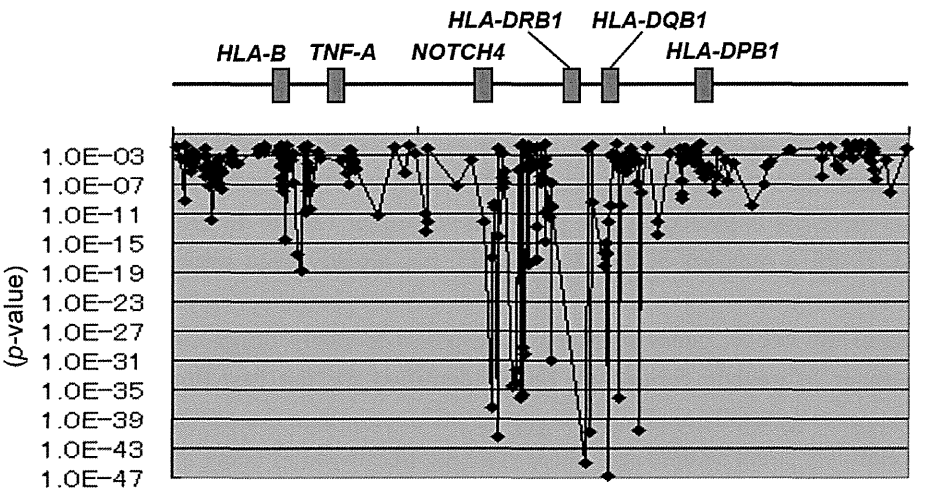
GWAS studies have been conducted for a number of diseases to date, and many of these have reported *HLA* as a susceptibility gene. In our own experience, narcolepsy [16], hepatitis B [29], rheumatoid arthritis [18], primary biliary cirrhosis [20], Stevens–Johnson syndrome, insulin autoimmune syndrome and type I diabetes have all shown strong association with certain *HLA* gene(s). Of these, narcolepsy, rheumatoid arthritis, primary biliary cirrhosis, type I diabetes and insulin autoimmune syndrome were associated most strongly with the *HLA-DR* and *HLA-DQ* regions, while hepatitis B and Stevens-Johnson syndrome were associated most strongly with the *HLA-DP* and *HLA-A* genes, respectively.

With regard to narcolepsy, Juji *et al.* [30] first reported in 1984 an extremely strong association with *HLA-DR2* (*HLA-DRB1*1501-DQB1*0602* haplotype according to the recent sequence-level nomenclature). We also found an extremely strong association between narcolepsy and the *HLA-DR/DQ* region with an SNP-based GWAS (Figure 3) [16]. If the results of HLA analysis in European and African populations are considered together, the primary susceptibility allele is assumed to be *HLA-DQB1*0602*.

Numerous GWAS have also been carried out for rheumatoid arthritis in Japan and elsewhere, and the *HLA-DR/DQ* region has been shown to have stronger association than any other region of the genome [18]. *HLA-DR4* has been known to be strongly associated with rheumatoid arthritis since the latter half of the 1970s; recent analysis at the sequence level has shown that *DRB1*0401* is most strongly associated in

European populations and *DRB1*0405* among Japanese. However, there are several other *DRB1* alleles that also exhibit susceptibility or resistance, and a hierarchy may be seen in their odds ratios.

Figure 3. GWAS confirmed the most strong association of the *HLA-DR/DQ* region with narcolepsy [16].



With primary biliary cirrhosis, also, the *HLA-DR/DQ* region showed the strongest association in the GWAS of European populations [31] and in the first GWAS of an Asian population [20]. From the analysis of *HLA* itself, *HLA-DRB1*0803-DQB1*0602* and *HLA-DRB1*0405-DQB1*0401* have been reported as susceptible haplotypes in the Japanese population [32], while *HLA-DRB1*0801-DQB1*04* was reported in European descendants [33].

4.2. Drug Hypersensitivity and HLA

There has also been great interest in *HLA* in its association with drug hypersensitivity. In 2002, it was reported that nearly 80% of patients who showed a hypersensitivity against the HIV drug, abacavir, possessed *HLA-B*5701*, with an odds ratio of 117 [34]. In 2004, a group from Taiwan found that of 44 patients with Stevens–Johnson syndrome induced by carbamazepine used for epilepsy seizures or as a psychotropic drug, all had *HLA-B*1502* [35]. However, less than 0.1% of Japanese possess *HLA-B*5701*, while *HLA-B*1502* is extremely rare. Consequently, it was predicted that the associations observed in the previous reports are hardly seen at all among Japanese.

In fact, Ozeki *et al.* [36] reported that adverse reactions in the skin as a result of carbamazepine are associated with *HLA-A*3101*. We reported independently that Stevens–Johnson syndrome/toxic epidermal necrolysis accompanied by eye manifestations caused by certain types of cold remedies is associated with *HLA-A*0206* [37]. Now, GWAS for this type of Stevens-Johnson syndrome has identified new susceptibility gene(s). Accordingly, GWAS can be powerful tool to investigate hypersensitivity to different kinds of drugs, and there is particular interest in associations with the *HLA* gene complex.

4.3. Characteristics of HLA and the Importance of HLA Typing

There are a number of unique characteristics of *HLA* genes and their polymorphisms, which indicates the limitation of SNP-based analysis and the importance of typing *HLA* genes themselves. First, the *HLA* genes are broadly classified into the Class I and Class II genes. Genes that exhibit high degrees of polymorphisms include *HLA-A*, *-B* and *-C* in Class I and *HLA-DRB1*, *-DQA1*, *-DQB1*, *-DPA1*, and *-DPB1* in Class II. Including *HLA* and non-HLA genes, a total of some 130 genes encoding proteins are densely located within a physical distance of about 4 Mbp on the short arm of Chromosome 6. They also show stronger linkage disequilibria than any other region of human genome. For these reasons, specifying a gene locus that is primarily associated with a disease is no easy task.

Second, commercially available genome-wide SNP typing arrays are unable to analyze the SNPs of the *HLA-DR* region. This is because there is copy number polymorphism of the *DRB* genes in the region: there are four functional *DRB* genes (*DRB1*, *B3*, *B4* and *B5*) and five pseudogenes *(DRB2*, *B6*, *B7*, *B8* and *B9*), and the gene composition differs depending upon the *DRB* haplotype. The SNPs of this region therefore do not conform to the Hardy–Weinberg equilibrium and, so, are not included on the arrays. Consequently, even though the *HLA-DQ* region may appear to show primary association from the results of an SNP-based GWAS, the adjacent *HLA-DR* region with extremely strong linkage disequilibrium must also be considered as a candidate region.

Third, genes in the Class II region are each adjacent on the genome as a pair, comprising an A gene and a B gene, and are linked to each other with a strong linkage disequilibrium. It is therefore very difficult to specify which gene of the pair is the primary one.

Fourth, as mentioned above, the *HLA* gene exhibits a high degree of polymorphism, and there are a huge number of alleles. There are almost no SNPs or SNP haplotypes that correspond one-on-one to individual *HLA* alleles. For example, more than 1300 alleles of *HLA-DRB1* have been admitted worldwide to date; for example, around 20 alleles with relatively high frequency and a great number of rare alleles have been found in the Japanese population; however, this sort of subclassification is not possible from SNP haplotypes.

Furthermore, a major feature is that a striking diversity between different populations can be observed. In other words, many *HLA* alleles are distributed only in certain regional populations.

Imputation of *HLA* alleles using *HLA* region SNP data is reported to have an accuracy of over 94% in European populations [38–40]. However, it is not perfect, especially for infrequent alleles, and the imputation is not yet fully available in Japanese or other Asian populations. The typing of the *HLA* genes is preferable for specifying *HLA* alleles directly involved in susceptibility, because there are multiple susceptibility alleles and resistance alleles, as well as 'neutral' alleles, and for many of these, the odds ratios are not consistent.

With regard to the *HLA*-associated diseases, therefore, detailed analysis, including the typing of the *HLA* genes themselves, are necessary to identify the primary *HLA* genes and alleles for each individual disease. These data will prove invaluable in clarifying the molecular mechanism through which HLA is associated with disease.

5. Conclusions and Issues for the Future

There are two hypotheses regarding the involvement of genome variation in common diseases: the common disease (common variants hypothesis and the common disease) and the rare variants hypothesis. In this regard, there is the argument that the common variants identified by GWAS as causing susceptibility to multifactorial diseases can only account for a small proportion of the genetic factors of disease, so that rare variants must also be important. This was symbolized by the term "missing heritability" [41], when only around 20 susceptibility loci for type II diabetes had been identified. Even in total, these could only explain about 5% of heritability. To date, over 60 common susceptibility loci have been identified, and this number is increasing all the time as a result of GWAS and meta-analyses carried out on greater scales. Further, it has been shown by the latest statistical analysis using all the GWAS data that around 40%–60% of all genetic factors can be explained. Therefore, it is assumed that there are still a great many relatively weak common susceptibility variants that have yet to be discovered.

To put it differently, we have not yet utilized the data obtained from GWAS to the fullest extent. For example, susceptibility genes that are not discovered by gathering samples from patients with the same disease name may be discovered by collecting detailed clinical data for each patient and then carrying out an analysis focused on clinical subsets. Considering a common disease from the viewpoint of its genetic architecture, the disease could be a collection of the many diseases that resemble each other, but also exhibit heterogeneity. Furthermore, it is likely that many susceptibility gene polymorphisms do not reach the so-called genome-wide significance level and, instead, exhibit moderate *p*-values. Establishing a method to identify the real susceptibility loci from this gray area is an issue that will need to be resolved in the future. It will be necessary to develop new methods that synthesize data from genetic ontology, pathway/network informatics and other fields and to establish statistical methods that can detect both intra-gene and inter-gene interactions. Our collaborators developed one such method that greatly improves the detection power of susceptibility loci [42].

Other than investigation by means of SNPs, there is also a need to clarify the degree to which variation, such as copy number variation (CNV) and short insertion/deletion variation, account for genetic factors in disease. Massive sequencing using next-generation sequencers is leading to astounding developments; to date, it has been very useful in identifying single genes responsible for hereditary diseases, and it has recently started to be applied to the search for susceptibility genes of multifactorial diseases. Until now, exome analysis has not turned up major results with respect to multifactorial disease. Considering that the majority of susceptibility SNPs identified by GWAS have been discovered in regions that regulate gene expression rather than in regions that code proteins, large-scale whole genome sequencing with a large number of patient and control samples may be needed. Then, the major challenge for the future is to establish a system to extract valuable data from the huge data produced by this new technology and to detect variants associated with certain multifactorial diseases.

HLA is already essential in clinical testing, such as organ and bone marrow transplantation and platelet transfusion. In addition, its association with over 100 types of diseases, including various autoimmune and inflammatory disorders, as well as infectious diseases, has been reported since the 1970s. Research aimed at understanding the mechanism of *HLA*-disease association commenced in the 1980s, but even now, the mechanism is not clearly known. In the 1990s, also, researchers carried out

many analyses of antigenic peptides eluted from *HLA* molecules prepared from mass cultured cells and analyses of T-cell clones created from patient samples, but were unable to gain a complete understanding of pathogenic peptides or the mechanisms of disease onset. It is hoped that there will be breakthroughs in the search for solutions to the huge riddle of disease mechanisms through advances, such as the diversity analysis of each *HLA* haplotype using next-generation sequencers, expression analysis of each *HLA* molecule using the latest protein chemistry and high-order structure analysis of the *HLA*-antigenic peptide-T-cell receptor complex.

Finally, the sharing of a huge amount of data produced by genome-wide variation analyses on various diseases through public databases, such as the Database of Genotypes and Phenotypes (dbGaP) [43], European Genome-Phenome Archive (EGA) [44] and GWAS Central [45], is crucial for the promotion of the complete identification of disease susceptibility genes and the understanding of the molecular mechanism of disease onset. We have also developed a public database for studies on the Japanese population [46–48].

Acknowledgments

This work was supported by grants-in-aid for scientific research from the Japanese Ministry of Education, Culture, Sports, Science and Technology, the Japanese Ministry of Health, Labor and Welfare and the Japan Science and Technology Agency.

Conflicts of Interest

The author declares no conflict of interest.

References

1. Risch, N. Searching for genetic determinants in the millennium. *Nature* **2000**, *405*, 847–856.
2. Ozaki, K.; Ohnishi, Y.; Iida, A.; Sekine, A.; Yamada, R.; Tsunoda, T.; Sato, H.; Hori, M.; Nakamura, Y.; Tanaka, T. Functional SNPs in the lymphotoxin-alpha gene that are associated with susceptibility to myocardial infarction. *Nat. Genet.* **2002**, *32*, 650–654.
3. Tamiya, G.; Shinya, M.; Imanishi, T.; Ikuta, T.; Makino, S.; Okamoto, K.; Furugaki, K.; Matsumoto, T.; Mano, S.; Ando, S.; *et al.* Whole genome association study of rheumatoid arthritis using 27039 microsatellites. *Hum. Mol. Genet.* **2005**, *14*, 2305–2321.
4. Database of Single Nucleotide Polymorphisms (dbSNP). Available online: http://www.ncbi.nlm.nih.gov/snp/ (accessed on 11 February 2014).
5. Database of HapMap Project. Available online: http://hapmap.ncbi.nlm.nih.gov/ (accessed on 11 February 2014).
6. Database of 1000 Genomes Project. Available online: http://www.1000genomes.org/ (accessed on 11 February 2014).
7. A Catalog of Published Genome-Wide Association Studies. Available online: http://www.genome.gov/gwastudies/ (accessed on 11 February 2014).

8. Sladek, R.; Rocheleau, G.; Rung, J.; Dina, C.; Shen, L.; Serre, D.; Boutin, P.; Vincent, D.; Belisle, A.; Hadjadj, S.; *et al.* Genome-wide association study identifies novel risk loci for type 2 diabetes. *Nature* **2007**, *445*, 881–885.
9. Saxena, R.; Voight, B.F.; Lyssenko, V.; Burtt, N.P.; de Bakker, P.I.; Chen, H.; Roix, J.J.; Kathiresan, S.; Hirschhorn, J.N.; Daly, M.J.; *et al.* Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels. *Science* **2007**, *316*, 1331–1336.
10. Scott, L.J.; Mohlke, K.L.; Bonnycastle, L.L.; Willer, C.J.; Li, Y.; Duren, W.L.; Erdos, M.R.; Stringham, H.M; Chines, P.S.; Jackson, A.U.; *et al.* A genome-wide association study of type 2 diabetes in Finns detects multiple susceptibility variants. *Science* **2007**, *316*, 1341–1345.
11. Wellcome Trust Case Control Consortium. Genome-wide association study of 14,000 cases of seven common diseases and 3000 shared controls. *Nature* **2007**, *447*, 661–678.
12. Yasuda, K.; Miyake, K.; Horikawa, Y.; Hara, K.; Osawa, H.; Furuta, H.; Hirota, Y.; Mori, H.; Jonsson, A.; Sato, Y.; *et al.* Variants in KCNQ1 are associated with susceptibility to type 2 diabetes mellitus. *Nat. Genet.* **2008**, *40*, 1092–1097.
13. Unoki, H.; Takahashi, A.; Kawaguchi, T.; Hara, K.; Horikoshi, M.; Andersen, G.; Ng, D.P.; Holmkvist, J.; Borch-Johnsen, K.; Jørgensen, T.; *et al.* SNPs in KCNQ1 are associated with susceptibility to type 2 diabetes in East Asian and European populations. *Nat. Genet.* **2008**, *40*, 1098–1102.
14. Miyake, K.; Horikawa, Y.; Hara, K.; Yasuda, K.; Osawa, H.; Furuta, H.; Hirota, Y.; Yamagata, K.; Hinokio, Y.; Oka, Y.; *et al.* Association of TCF7L2 polymorphisms with susceptibility to type 2 diabetes in 4,087 Japanese subjects. *J. Hum. Genet.* **2008**, *53*, 174–180.
15. Matsuki, K.; Juji, T.; Tokunaga, K.; Naohara, T.; Satake, M.; Honda, Y. Human histocompatibility leukocyte antigen (HLA) haplotype frequencies estimated from the data on HLA class I, II, and III antigens in 111 Japanese narcoleptics. *J. Clin. Invest.* **1985**, *76*, 2078–2083.
16. Miyagawa, T.; Kawashima, M.; Nishida, N.; Ohashi, J.; Kimura, R.; Fujimoto, A.; Shimada, M.; Morishita, S.; Shigeta, T.; Lin, L.; *et al.* Variant between CPT1B and CHKB associated with susceptibility to narcolepsy. *Nat. Genet.* **2008**, *40*, 1324–1328.
17. Mahasirimongkol, S.; Yanai, H.; Mushiroda, T.; Promphittayarat, W.; Wattanapokayakit, S.; Phromjai, J.; Yuliwulandari, R.; Wichukchinda, N.; Yowang, A.; Yamada, N.; *et al.* Genome-wide association studies of tuberculosis in Asians identify distinct at-risk locus foe young tuberculosis. *J. Hum. Genet.* **2012**, *57*, 363–367.
18. Okada, Y.; Terao, C.; Ikari, K.; Kochi, Y.; Ohmura, K.; Suzuki, A.; Kawaguchi, T.; Stahl, E.A.; Kurreeman, F.A.; Nishida, N.; *et al.* Meta-analysis of genome-wide association studies identifies multiple novel loci associated with rheumatoid arthritis in the Japanese population. *Nat. Genet.* **2012**, *44*, 511–516.
19. Takamoto, M.; Kaburaki, T.; Mabuchi, A.; Araie, M.; Amano, S.; Aihara, M.; Tomidokoro, A.; Iwase, A.; Mabuchi, F.; Kashiwagi, K.; *et al.* Common variants on chromosome 9q21 are associated with normal tension glaucoma. *PLoS One* **2012**, *7*, e40107.
20. Nakamura, M.; Nishida, N.; Kawashima, M.; Aiba, Y.; Tanaka, A.; Yasunami, M.; Nakamura, H.; Komori, A.; Nakamuta, M.; Zeniya, M.; *et al.* Genome-wide association study identifies TNFSF15 and POU2AF1 as susceptibility loci for primary biliary cirrhosis in the Japanese population. *Am. J. Hum. Genet.* **2012**, *91*, 721–728.

21. Okamoto, K.; Tokunaga, K.; Doi, K.; Fujita, T.; Suzuki, H.; Katoh, T.; Watanabe, T.; Nishida, N.; Mabuchi, A.; Takahashi, A.; *et al.* Common variation in GPC5 is associated with acquired nephrotic syndrome. *Nat. Genet.* **2011**, *43*, 459–463.
22. Cotsapas, C.; Voight, B.F.; Rossin, E.; Lage, K.; Neale, B.M.; Wallace, C.; Abecasis, G.R.; Barrett, J.C.; Behrens, T.; Cho, J.; *et al.* Pervasive sharing of genetic effects in autoimmune disease. *PLoS Genet.* **2011**, *7*, e1002254.
23. Parkes, M.; Cortes, A.; van Heel, D.A.; Brown, M.A. Genetic insights into common pathways and complex relationships among immune-mediated diseases. *Nat. Rev. Genet.* **2013**, *14*, 661–673.
24. Miyagawa, T.; Miyadera, H.; Tanaka, S.; Kawashima, M.; Shimada, M.; Honda, Y.; Tokunaga, K.; Honda, M. Abnormally low serum acylcarnitine level in narcolepsy. *Sleep* **2011**, *34*, 349–353.
25. Miyagawa, T.; Kawamura, H.; Obuchi, M.; Ikesaki, A.; Ozaki, A.; Tokunaga, K.; Inoue, Y.; Honda, M. Effects of oral L-carnitine administration in narcolepsy patients: A randomized, double-blind, cross-over and placebo-controlled trial. *PLoS One* **2013**, *8*, e53707.
26. Hallmayer, J.; Faraco, J.; Lin, L.; Hesselson, S.; Winkelmann, J.; Kawashima, M.; Mayer, G.; Plazzi, G.; Nevsimalova, S.; Bourgin, P.; *et al.* Narcolepsy is strongly associated with the TCR alpha locus. *Nat. Genet.* **2009**, *41*, 708–711.
27. Kornum, B.R.; Kawashima, M.; Faraco, J.; Lin, L.; Rico, T.J.; Hesselson, S.; Axtell, R.C.; Kuipers, H.; Weiner, K.; Hamacher, A.; *et al.* Common variants in P2RY11 are associated with narcolepsy. *Nat. Genet.* **2011**, *43*, 66–71.
28. Tanaka, Y.; Nishida, N.; Sugiyama, M.; Kurosaki, M.; Matsuura, K.; Sakamoto, N.; Nakagawa, M.; Korenaga, M.; Hino, K.; Hige, S.; *et al.* Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. *Nat. Genet.* **2009**, *41*, 1105–1109.
29. Nishida, N.; Sawai, H.; Matsuura, K.; Sugiyama, M.; Ahn, S.H.; Park, J.Y.; Hige, S.; Kang, J.H.; Suzuki, K.; Kurosaki, M.; *et al.* Genome-wide association study confirming association of *HLA-DP* with protection against chronic hepatitis B and viral clearance in Japanese and Korean. *PLoS One* **2012**, *7*, e39175.
30. Juji, T.; Satake, M.; Honda, Y.; Doi, Y. *HLA* antigens in Japanese patients with narcolepsy. All patients were DR2 positive. *Tissue Antigens* **1984**, *24*, 316–319.
31. Hirschfield, G.M.; Liu, X.; Xu, C.; Lu, Y.; Xie, G.; Lu, Y.; Gu, X.; Walker, E.J.; Jing, K.; Juran, B.D.; *et al.* Primary biliary cirrhosis associated with *HLA*, *IL12A*, and *IL12RB2* variants. *N. Engl. J. Med.* **2009**, *360*, 2544–2555.
32. Nakamura, M.; Yasunami, M.; Kondo, H.; Horie, H.; Aiba, Y.; Komori, A.; Migita, K.; Yatsuhashi, H.; Ito, M.; Shimoda, S.; *et al.* Analysis of *HLA-DRB1* polymorphisms in Japanese patients with primary biliary cirrhosis (PBC): The *HLA-DRB1* polymorphism determines the relative risk of antinuclear antibodies for disease progression in PBC. *Hepatol. Res.* **2010**, *40*, 494–504.
33. Begovich, A.B.; Klitz, W.; Moonsamy, P.V.; van de Water, J.; Peltz, G.; Gershwin, M.E. Genes within the *HLA* class II region confer both predisposition and resistance to primary biliary cirrhosis. *Tissue Antigens* **1994**, *43*, 71–77.

34. Mallal, S.; Nolan, D.; Witt, C.; Masel, G.; Martin, A.M.; Moore, C.; Sayer, D.; Castley, A.; Mamotte, C.; Maxwell, D.; *et al.* Association between presence of *HLA-B**5701, *HLA-DR7* and *HLA-DQ3* and hypersensitivity to HIV-1 reverse-transcriptase inhibitor abacavir. *Lancet* **2002**, *359*, 727–732.
35. Chung, W.H.; Hung, S.I.; Hong, H.S.; Hsih, M.S.; Yang, L.C.; Ho, H.C.; Wu, J.Y.; Chen, Y.T. Medical genetics: A marker for Stevens-Johnson syndrome. *Nature* **2004**, *428*, 486.
36. Ozeki, T.; Mushiroda, T.; Yowang, A.; Takahashi, A.; Kubo, M.; Shirakata, Y.; Ikezawa, Z.; Iijima, M.; Shiohara, T.; Hashimoto, K.; *et al.* Genome-wide association study identifies *HLA-A*3101* allele as a genetic risk factor for carbamazepine-induced cutaneous adverse drug reactions in Japanese population. *Hum. Mol. Genet.* **2011**, *20*, 1034–1041.
37. Ueta, M.; Tokunaga, K.; Sotozono, C.; Inatomi, T.; Yabe, T.; Matsushita, M.; Mitsuishi, Y.; Kinoshita, S. *HLA* class Ⅰ and Ⅱ gene polymorphisms in Stevens-Johnson syndrome with ocular complications in Japanese. *Mol. Vis.* **2008**, *14*, 550–555.
38. Dilthey, A.; Leslie, S.; Moutsianas, L.; Shen, J.; Cox, C.; Nelson, M.R.; McVean, G. Multi-population classical *HLA* type imputation. *PLoS Comput. Biol.* **2013**, *9*, e1002877.
39. Zheng, X.; Shen, J.; Cox, C.; Wakefield, J.C.; Ehm, M.G.; Nelson, M.R.; Weir, B.S. *HIBAG-HLA* genotype imputation with attribute bagging. *Pharmacogenomics J.* **2013**, doi:10.1038/tpj.2013.18.
40. Jia, X.; Han, B.; Onengut-Gumuscu, S.; Chen, W.M.; Concannon, P.J.; Rich, S.S.; Raychaudhuri, S.; de Bakker, P.I. Imputing amino acid po*lymorphisms in hum*an leucocyte antigens. *PLoS One* **2013**, *8*, e64683.
41. Maher, B. Personal genomes: The case of the missing heritability. *Nature* **2008**, *456*, 18–21.
42. Dinu, I.; Mahasirimongkol, S.; Liu, Q.; Yanai, H.; El-Din, N.S.; Kreiter, E.; Wu, X.; Jabbari, S.; Tokunaga, K.; Yasui, Y. SNP-SNP interactions discovered by logic regression explain Crohn's disease genetics. *PLoS One* **2012**, *7*, e43035.
43. Database of Genotypes and Phenotypes (dbGaP). Available online: http://www.ncbi.nlm.nih.gov/gap/ (accessed on 11 February 2014).
44. European Genome-Phenome Archive (EGA). Available online: https://www.ebi.ac.uk/ega/ (accessed on 11 February 2014).
45. GWAS Central. Available online: https://www.gwascentral.org/ (accessed on 11 February 2014).
46. Koike, A.; Nishida, N.; Inoue, I.; Tsuji, S.; Tokunaga, K. Genome-wide association database developed in the Japanese Integrated Database Project. *J. Hum. Genet.* **2009**, *54*, 543–546.
47. Koike, A.; Nishida, N.; Yamashita, D.; Tokunaga, K. Comparative analysis of copy number variation detection methods and database construction. *BMC Genet.* **2011**, *12*, e29.
48. Human Genome Variation Database. Available online: https://gwas.biosciencedbc.jp/index.html/ (accessed on 11 February 2014).

OPEN ACCESS Freely available online

PLOS | ONE

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

Nao Nishida[1,2*9], Hiromi Sawai[2,9], Koichi Kashiwase[3], Mutsuhiko Minami[3], Masaya Sugiyama[1], Wai-Kay Seto[4], Man-Fung Yuen[4], Nawarat Posuwan[5], Yong Poovorawan[5], Sang Hoon Ahn[6], Kwang-Hyub Han[6], Kentaro Matsuura[7], Yasuhito Tanaka[7], Masayuki Kurosaki[8], Yasuhiro Asahina[9,10], Namiki Izumi[8], Jong-Hon Kang[11], Shuhei Hige[12], Tatsuya Ide[13], Kazuhide Yamamoto[14], Isao Sakaida[15], Yoshikazu Murawaki[16], Yoshito Itoh[17], Akihiro Tamori[18], Etsuro Orito[19], Yoichi Hiasa[20], Masao Honda[21], Shuichi Kaneko[21], Eiji Mita[22], Kazuyuki Suzuki[23], Keisuke Hino[24], Eiji Tanaka[25], Satoshi Mochida[26], Masaaki Watanabe[27], Yuichiro Eguchi[28], Naohiko Masaki[1], Kazumoto Murata[1], Masaaki Korenaga[1], Yoriko Mawatari[1], Jun Ohashi[29], Minae Kawashima[2], Katsushi Tokunaga[2], Masashi Mizokami[1]*

1 The Research Center for Hepatitis and Immunology, National Center for Global Health and Medicine, Ichikawa, Chiba, Japan, **2** Department of Human Genetics, Graduate School of Medicine, The University of Tokyo, Bunkyo-ku, Tokyo, Japan, **3** HLA Laboratory, Japanese Red Cross Kanto-Koshinetsu Block Blood Center, Koutou-ku, Tokyo, Japan, **4** Department of Medicine, Queen Mary Hospital, Hong Kong, **5** Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand, **6** Department of Internal Medicine, Yonsei University College of Medicine, Seoul, South Korea, **7** Department of Virology & Liver Unit, Nagoya City University Graduate School of Medical Sciences, Nagoya, Aichi, Japan, **8** Division of Gastroenterology and Hepatology, Musashino Red Cross Hospital, Musashino, Tokyo, Japan, **9** Department of Liver Disease Control, Tokyo Medical and Dental University, Bunkyo-ku, Tokyo, Japan, **10** Department of Gastroenterology and Hepatology, Tokyo Medical and Dental University, Bunkyo-ku, Tokyo, Japan, **11** Department of Internal Medicine, Teine Keijinkai Hospital, Sapporo, Hokkaido, Japan, **12** Department of Internal Medicine, Hokkaido University Graduate School of Medicine, Sapporo, Hokkaido, Japan, **13** Division of Gastroenterology, Department of Medicine, Kurume University School of Medicine, Kurume, Fukuoka, Japan, **14** Department of Gastroenterology and Hepatology, Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Okayama, Okayama, Japan, **15** Gastroenterology and Hepatology, Yamaguchi University Graduate School of Medicine, Ube, Yamaguchi, Japan, **16** Faculty of Medicine, Tottori University, Tottori, Tottori, Japan, **17** Molecular Gastroenterology and Hepatology, Kyoto Prefectural University of Medicine, Kyoto, Kyoto, Japan, **18** Department of Hepatology, Osaka City University Graduate School of Medicine, Osaka, Osaka, Japan, **19** Department of Gastroenterology, Nagoya Daini Red Cross Hospital, Nagoya, Aichi, Japan, **20** Department of Gastroenterology and Metabology, Ehime University Graduate School of Medicine, Toon, Ehime, Japan, **21** Department of Gastroenterology, Kanazawa University Graduate School of Medicine, Kanazawa, Ishikawa, Japan, **22** Department of Gastroenterology and Hepatology, National Hospital Organization Osaka National Hospital, Osaka, Osaka, Japan, **23** Division of Gastroenterology and Hepatology, Department of Internal Medcine, Iwate Medical University, Morioka, Iwate, Japan, **24** Division of Hepatology and Pancreatology, Kawasaki Medical College, Kurashiki, Okayama, Japan, **25** Department of Medicine, Shinshu University School of Medicine, Matsumoto, Nagano, Japan, **26** Division of Gastroenterology and Hepatology, Saitama Medical University, Iruma, Saitama, Japan, **27** Department of Gastroenterology, Kitasato University School of Medicine, Sagamihara, Kanagawa, Japan, **28** Department of Internal Medicine, Saga Medical School, Saga, Saga, Japan, **29** Faculty of Medicine, University of Tsukuba, Tsukuba, Ibaraki, Japan

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.

Citation: Nishida N, Sawai H, Kashiwase K, Minami M, Sugiyama M, et al. (2014) 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. doi:10.1371/journal.pone.0086449

Editor: Ferruccio Bonino, University of Pisa, Italy

Received November 13, 2013; **Accepted** December 10, 2013; **Published** February 10, 2014

Funding: This work was supported by a Grant-in-Aid from the Ministry of Health, Labour, and Welfare of Japan H24-Bsou-kanen-ippan-011 and H24-kanen-ippan-004 to Masashi Mizokami, H23-kanen-005 to Katsushi Tokunaga, H25-kanen-wakate-013 to Nao Nishida, and H25-kanen-wakate-012 to Hiromi Sawai. This work was also supported by The Grant for National Center for Global Health and Medicine 22-shi-302 to Masashi Mizokami and 24-shi-107 to Nao Nishida. Partial support by Grant-in-Aid from the Ministry of Education, Culture, Sports, Science of Japan [grant number 22133008] for Scientific Research on Innovative Areas to Katsushi Tokunaga, [grant number 24790728] for Young Scientists (B) to Nao Nishida, and [grant number 25870178] for Young Scientists (B) to Hiromi Sawai, is also acknowledged. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: mmizokami@hospk.ncgm.go.jp (MM); nishida-75@umin.ac.jp (NN)

These authors contributed equally to this work.

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.

doi:10.1371/journal.pone.0086449.t001

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.

doi:10.1371/journal.pone.0086449.t002

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: $\text{logit}(p) = -3.905+0.083*\text{age}+(-0.929)*\text{sex}+(-0.684)*$*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)

Acknowledgments

We would like to thank all the patients and families who contributed to the study. We are also grateful to Ms. Mayumi Ishii (National Center for Global Health and Medicine), Ms. Megumi Sageshima, Yuko Hirano, Natsumi Baba, Rieko Shirahashi, Ayumi Nakayama (University of Tokyo), and Yuko Ohara (Japanese Red Cross Kanto-Koshinetsu Block Blood Center) for technical assistance.

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.

References

1. Chen DS (1993) From hepatitis to hepatoma: lessons from type B viral hepatitis. Science 262: 369–370.
2. Custer B, Sullivan SD, Hazlet TK, Iloeje U, Veenstra DL, et al. (2004) Global epidemiology of hepatitis B virus. J Clin Gastroenterol 38: S158–168.
3. Zidan A, Scheuerlein H, Schule S, Settmacher U, Rauchfuss F (2012) Epidemiological pattern of hepatitis B and hepatitis C as etiological agents for hepatocellular carcinoma in iran and worldwide. Hepat Mon 12: e6894.
4. Pungpapong S, Kim WR, Poterucha JJ (2007) Natural history of hepatitis B virus infection: an update for clinicians. Mayo Clin Proc 82: 967–975.
5. Kim DW, Lee SA, Hwang ES, Kook YH, Kim BJ (2012) Naturally occurring precore/core region mutations of hepatitis B virus genotype C related to hepatocellular carcinoma. PLoS One 7: e47372.
6. Jiang DK, Sun J, Cao G, Liu Y, Lin D, et al. (2013) Genetic variants in STAT4 and HLA-DQ genes confer risk of hepatitis B virus-related hepatocellular carcinoma. Nat Genet 45: 72–75.
7. Kamatani Y, Wattanapokayakit S, Ochi H, Kawaguchi T, Takahashi A, et al. (2009) A genome-wide association study identifies variants in the HLA-DP locus associated with chronic hepatitis B in Asians. Nat Genet 41: 591–595.
8. Mbarek H, Ochi H, Urabe Y, Kumar V, Kubo M, et al. (2011) A genome-wide association study of chronic hepatitis B identified novel risk locus in a Japanese population. Hum Mol Genet 20: 3884–3892.
9. Nishida N, Sawai H, Matsuura K, Sugiyama M, Ahn SH, et al. (2012) Genome-wide association study confirming association of HLA-DP with protection against chronic hepatitis B and viral clearance in Japanese and Korean. PLoS One 7: e39175.
10. Guo X, Zhang Y, Li J, Ma J, Wei Z, et al. (2011) Strong influence of human leukocyte antigen (HLA)-DP gene variants on development of persistent chronic hepatitis B virus carriers in the Han Chinese population. Hepatology 53: 422–428.
11. An P, Winkler C, Guan L, O'Brien SJ, Zeng Z (2011) A common HLA-DPA1 variant is a major determinant of hepatitis B virus clearance in Han Chinese. J Infect Dis 203: 943–947.
12. Li J, Yang D, He Y, Wang M, Wen Z, et al. (2011) Associations of HLA-DP variants with hepatitis B virus infection in southern and northern Han Chinese populations: a multicenter case-control study. PLoS One 6: e24221.
13. Vermehren J, Lotsch J, Susser S, Wicker S, Berger A, et al. (2012) A common HLA-DPA1 variant is associated with hepatitis B virus infection but fails to distinguish active from inactive Caucasian carriers. PLoS One 7: e32605.
14. Sawai H, Nishida N, Mbarek H, Matsuda K, Mawatari Y, et al. (2012) No association for Chinese HBV-related hepatocellular carcinoma susceptibility SNP in other East Asian populations. BMC Med Genet 13: 47.
15. Chandanayingyong D, Stephens HA, Fan L, Sirikong M, Longta P, et al. (1994) HLA-DPB1 polymorphism in the Thais of Southeast Asia. Hum Immunol 40: 20–24.

16. Chandanayingyong D, Stephens HA, Klaythong R, Sirikong M, Udee S, et al. (1997) HLA-A, -B, -DRB1, -DQA1, and -DQB1 polymorphism in Thais. Hum Immunol 53: 174–182.
17. Maneemaroj R, Stephens HA, Chandanayingyong D, Longta K, Bejrachandra S (1997) HLA class II allele frequencies in northern Thais (Kamphaeng Phet). J Med Assoc Thai 80 Suppl 1: S20–24.
18. Wu TW, Chu CC, Ho TY, Chang Liao HW, Lin SK, et al. (2013) Responses to booster hepatitis B vaccination are significantly correlated with genotypes of human leukocyte antigen (HLA)-DPB1 in neonatally vaccinated adolescents. Hum Genet.
19. Hu L, Zhai X, Liu J, Chu M, Pan S, et al. (2012) Genetic variants in human leukocyte antigen/DP-DQ influence both hepatitis B virus clearance and hepatocellular carcinoma development. Hepatology 55: 1426–1431.
20. Li S, Qian J, Yang Y, Zhao W, Dai J, et al. (2012) GWAS identifies novel susceptibility loci on 6p21.32 and 21q21.3 for hepatocellular carcinoma in chronic hepatitis B virus carriers. PLoS Genet 8: e1002791.
21. Stephens M, Smith NJ, Donnelly P (2001) A new statistical method for haplotype reconstruction from population data. Am J Hum Genet 68: 978–989.

Risk Factors for Long-Term Persistence of Serum Hepatitis B Surface Antigen Following Acute Hepatitis B Virus Infection in Japanese Adults

Kiyoaki Ito,[1,13*] Hiroshi Yotsuyanagi,[2*] Hiroshi Yatsuhashi,[3] Yoshiyasu Karino,[4] Yasuhiro Takikawa,[5] Takafumi Saito,[6] Yasuji Arase,[7] Fumio Imazeki,[8] Masayuki Kurosaki,[9] Takeji Umemura,[10] Takafumi Ichida,[11] Hidenori Toyoda,[12] Masashi Yoneda,[13] Eiji Mita,[14] Kazuhide Yamamoto,[15] Kojiro Michitaka,[16] Tatsuji Maeshiro,[17] Junko Tanuma,[18] Yasuhito Tanaka,[19] Masaya Sugiyama,[1] Kazumoto Murata,[1] Naohiko Masaki,[1] and Masashi Mizokami,[1] and the Japanese AHB Study Group

The proportion of patients who progress to chronicity following acute hepatitis B (AHB) varies widely worldwide. Moreover, the association between viral persistence after AHB and hepatitis B virus (HBV) genotypes in adults remains unclear. A nationwide multicenter study was conducted throughout Japan to evaluate the influence of clinical and virological factors on chronic outcomes in patients with AHB. For comparing factors between AHB patients with viral persistence and those with self-limited infection, 212 AHB patients without human immunodeficiency virus (HIV) coinfection were observed in 38 liver centers until serum hepatitis B surface antigen (HBsAg) disappeared or a minimum of 6 months in cases where HBsAg persisted. The time to disappearance of HBsAg was significantly longer for genotype A patients than that of patients infected with non-A genotypes. When chronicity was defined as the persistence of HBsAg positivity for more than 6 or 12 months, the rate of progression to chronicity was higher in patients with genotype A, although many cases caused by genotype A were prolonged cases of AHB, rather than chronic infection. Multivariate logistic regression analysis revealed only genotype A was independently associated with viral persistence following AHB. A higher peak level of HBV DNA and a lower peak of alanine aminotransferase (ALT) levels were characteristics of AHB caused by genotype A. Treatment with nucleotide analogs (NAs) did not prevent progression to chronic infection following AHB overall. Subanalysis suggested early NA initiation may enhance the viral clearance. *Conclusion*: Genotype A was an independent risk factor for progression to chronic infection following AHB. Our data will be useful in elucidating the association between viral persistence after AHB, host genetic factors, and treatment with NAs in future studies. (HEPATOLOGY 2014;59:89-97)

Abbreviations: AHB, acute hepatitis B; ALT, alanine aminotransferase; anti-HBc, antibody to hepatitis B core antigen; anti-HBs, antibody to HBsAg; HBeAg, hepatitis B e-antigen; CLIA, chemiluminescent enzyme immunoassay; EIA, enzyme immunoassay; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HIV, human immunodeficiency virus; IgM, immunoglobulin M; anti-HBe, antibody to HBeAg; NAs, nucleotide analogs; RPHA, reverse passive hemagglutination.

From the [1]The Research Center for Hepatitis and Immunology, National Center for Global Health and Medicine, Ichikawa, Japan; [2]Department of Internal Medicine, Graduate School of Medicine, University of Tokyo, Tokyo, Japan; [3]Clinical Research Center, NHO Nagasaki Medical Center, Nagasaki, Japan; [4]Department of Gastroenterology, Sapporo Kosei General Hospital, Sapporo; [5]First Department of Internal Medicine, Iwate Medical University, Morioka, Japan; [6]Department of Gastroenterology, Yamagata University School, Yamagata, Japan; [7]Department of Hepatology, Toranomon Hospital, Tokyo, Japan; [8]Department of Medicine and Clinical Oncology, Chiba University, Graduate School of Medicine, Chiba, Japan; [9]Division of Gastroenterology and Hepatology, Musashino Red Cross Hospital, Tokyo, Japan; [10]Department of Medicine, Shinshu University School of Medicine, Matsumoto, Japan; [11]Department of Gastroenterology and Hepatology, Juntendo University Shizuoka Hospital, Shizuoka, Japan; [12]Department of Gastroenterology, Ogaki Municipal Hospital, Ogaki, Japan; [13]Department of Gastroenterology, Aichi Medical University School of Medicine, Nagakute, Japan; [14]National Hospital Organization Osaka National Hospital, Osaka, Japan; [15]Department of Gastroenterology and Hepatology, Okayama University Graduate School of Medicine, Okayama, Japan; [16]Department of Gastroenterology, Ehime University Graduate School of Medicine, Toon, Japan; [17]First Department of Internal Medicine, University Hospital, Faculty of Medicine, University of the Ryukyu, Okinawa, Japan; [18]AIDS Clinical Center, National Center for Global Health and Medicine, Tokyo, Japan; [19]Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan.

Received January 5, 2013; accepted July 10, 2013.

Hepatitis B virus (HBV) infection is one of the most common persistent viral infections in humans. Approximately 2 billion people worldwide have been exposed to HBV and 350 million of them remain persistently infected.[1] The incidence of HBV infection and the patterns of transmission vary greatly worldwide among different population subgroups.[2] In Western countries, chronic HBV infection is relatively rare and acquired primarily in adulthood by way of sexual transmission or the use of injectable drugs. Meanwhile, in Asia and most of Africa, the majority of infections are the result of transmission from an infected mother to her newborn. However, very few studies of acute hepatitis B (AHB) in adults have been reported.

HBV genomic sequences vary worldwide and have been classified into at least eight genotypes (A through H) based on an intergroup divergence of 8% or more over the complete nucleotide sequence.[3,4] These genotypes have distinct geographic distributions.[5-7] In particular, genotype A is predominant in Northwestern Europe, the United States, Central Africa, and India.[8, 9] The Japanese have been infected with genotypes B and C since prehistoric times.[10] Recently, many lines of evidence have revealed among the Japanese an increase in acute infection with HBV genotype A following sexual transmission.[11,12] As a result of this increasing transmission of genotype A, the distribution of HBV genotypes in Japan clearly differs among patients with acute and chronic infections.[13] Moreover, recent studies suggest that acute infection with HBV genotype A may be associated with an increased risk of progression to persistent infection.[15] Indeed, the prevalence of HBV genotype A in chronic hepatitis B patients doubled in Japan between 2000-2001 and 2005-2006 (1.7% versus 3.5%).[11]

Human immunodeficiency virus (HIV)-1 infection results in an immunodeficient state, with the virus sharing routes of transmission with HBV. HIV-related immunodepletion influences the natural history of HBV infection, and epidemiological studies have revealed that HIV-positive patients are more likely to have a prolonged acute illness following HBV infection and lower rates of hepatitis B e-antigen (HBeAg) clearance.[16] Therefore, in this study patients with coinfection of HIV were excluded to examine the influence of HBV genotype directly without the confounding influence of HIV.

From 2005 to 2010, a multicenter cohort study was conducted throughout Japan on 212 patients with AHB. The aim of this cohort study was to assess the influence of clinical and virological factors, including HBV genotypes and treatment with nucleotide analogs (NAs), on AHB patients who became persistently infected.

Patients and Methods

Patients With AHB. The multiple-source cohort included 212 randomly selected AHB patients without coinfection of HIV. From 2005 through 2010, the study participants were recruited from 38 liver centers throughout Japan. The cohort included patients who were admitted to the hospital because of AHB and who visited the hospital every month after being discharged. The diagnosis of AHB was contingent on the rapid onset of clinical symptoms accompanied by elevated serum alanine aminotransferase (ALT) levels, the detection of serum hepatitis B surface antigen (HBsAg), and a high-titer antibody to hepatitis B core antigen (anti-HBc) of the immunoglobulin M (IgM) class. Patients with initial high-titer anti-HBc (>10.0 S/CO) were diagnosed as having an exacerbation of chronic hepatitis B and were excluded. If the patient had been tested previously, the absence of serum HBsAg and anti-HBc before admission was verified from the medical record to discriminate a new infection from an acute exacerbation of a persistent infection. Patients with acute hepatitis A, hepatitis C, and drug- or alcohol-induced acute hepatitis were also excluded; hepatitis D virus infection was not determined because of its extreme rarity in Japan. The study protocol conformed to the 1975 Declaration of

Supported by Japanese Ministry of Health, Labor and Welfare grant H21-003 and Japanese Ministry of Education, Culture, Sports, Science and Technology grant 22790679.

**These authors contributed equally to this work.*

Address reprint requests to: Masashi Mizokami, M.D., Ph.D., Research Center for Hepatitis and Immunology, National Center for Global Health and Medicine, 1-7-1, Konodai, Ichikawa 272-8516, Japan. E-mail:mmizokami@hospk.ncgm.go.jp; fax: +81-(0)47-375-4766.

View this article online at wileyonlinelibrary.com.

DOI 10.1002/hep.26635

Potential conflict of interest: Nothing to report.

Additional Supporting Information may be found in the online version of this article.