［課程－２］

審査の結果の要旨

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Hepatitis, an inflammation of the liver cells and damage to the liver, is a serious global health issue with different types and causes, but similar symptoms. If left untreated, it can develop into chronic (long-term) hepatitis, liver failure, hepatocellular carcinoma (HCC) and even death. Based on the World Health Organization, an estimated 1.4 million deaths occur annually from acute infection and hepatitis-related liver cirrhosis and cancer. Approximately 47% of hepatitis-related deaths are attributable to hepatitis B virus (HBV), 48% to hepatitis C virus (HCV), and the remainder to hepatitis A and hepatitis E viruses. Not all of those who have acute infection with HBV and HCV develop hepatitis-related diseases, leading to the speculation that there may be genetic factors involved in the host susceptibility to viral hepatitis-related diseases.

The current study consists of two parts. The first part was a search for yet unidentified host genetic factors affecting susceptibility to HCV-associated HCC in a Japanese population. The case group of the discovery sample set included hepatitis C patients who eradicated HCV by interferon-based (IFN-based) therapy and achieved sustained virologic response (SVR) and developed HCC ≥ 1 year after the end of treatment (EOT), and the control group of the discovery sample set consisted of hepatitis C patients who eradicated HCV by IFN-based therapy and achieved SVR but did not develop HCC ≥ 5 years after EOT. For this part, whole-genome imputation method was conducted on genotyped SNPs obtained from Affymetrix Axiom Genome-Wide ASI 1 array. After performing a data quality control, genome-wide association study (GWAS) was conducted using logistic regression test adjusting for gender. The association of SNPs with p-value < 1.0E-04 with HCV-associated HCC was analyzed in an independent Japanese sample set (replication set) consisting of case group with criteria same as case group in the discovery sample set and control group that included hepatitis C patients who eradicated HCV by IFN-based therapy and achieved SVR but did not develop HCC ≥ 3 years after EOT. In addition, as the ratio of male-to-female deviated from 1:1, I performed analysis of association of SNPs located on X chromosome. Finally, a gene-based and gene ontology (GO) enrichment analysis were conducted. The results of first part are summarized as follows:

1. GWAS of whole-genome imputation data revealed loci with suggestive level of significance (P < 1.0E-04) associated with HCV-associated HCC. One locus (rs9459344, p-value = 9.42E-09, OR = 1.94) showed genome-wide significance (P < 5.0E-08) and some other loci showed suggestive level of significance ($P < 1 \times 10^{-4}$). For 96 SNPs (genotyped and imputed) with p-value less than 1.0E-04, the validation of 74 SNPs in the discovery set was successfully

conducted. After performing quality control, 69 SNPs were retained for association analysis in the replication set using a logistic regression test adjusting for the gender. However, none of those 69 SNPs p-values passed the Bonferroni correction (7.2E-04).

2. The association of SNPs located on X chromosome with HCV-associated HCC was analyzed using XWAS software. After performing X-chromosome specific quality control, association analysis was conducted in males and females using Fischer's exact test and final p-values were produced by combining the results of two tests. None of p-values of SNPs located on X chromosome passed the significance threshold (Bonferroni correction: 4.1E-07).
3. Using the results of GWAS, a gene-based and GO enrichment analyses showed a number of genes and GO processes significantly associated with HCV-associated HCC. The *RASGEF1B* gene, a protein-coding gene, with 27 flanking SNPs in GWAS results was found to be the most significantly associated gene (p-value = 6.0E-05). Among GO processes, the most highly enriched GO process was "regulation of MAP kinase activity" (p-value = 1.75E-06) with 14 genes listed in gene-based association results represented in the process.

The second part of this study was a GWAS conducted in 329 chronic hepatitis B (CHB) cases and 320 healthy control obtained from the Thai population. Whole-genome imputation method was also conducted on genotyped SNPs obtained from Affymetrix Axiom Genome-Wide ASI 1 array and GWAS of whole-genome imputation data was conducted. Imputation of alleles of *HLA-DPA1*, *HLA-DPB1*, and *HLA-DQB1* genes were conducted based on SNP data produced by GWAS array and validated in samples and analysis of association of HLA alleles with susceptibility/resistance to CHB was performed using Pearson's chi-squared test. The results of the second part are summarized as follows:

1. The association analysis of typed and imputed data identified 1 locus on chromosome 6 (*HLA-DPA1/DPB1*) that passed the genome-wide level of significance (P < 5.0E-08). Logistic regression analysis conditioning on the top SNP in this region (rs7770370, P = 8.6E-10, OR = 0.49) did not detect any other genes in the HLA region significantly associated with CHB in Thai.
2. In addition, 4 more novel candidate loci on chromosomes 3, 4, 10, and 12 showed suggestive level of significance (rs1061307, located on 5' UTR of *PLSCR1* gene on chromosome 3, p-value = 5.92E-06, OR = 0.47; rs62321986, located 28kb 5' flanking region of *PDLIM5* gene on chromosome 4, p-value = 7.69-E06, OR = 2.5; rs144998273, located in the intron of *SGPL1* gene on chromosome 10, p-value = 4.65E-06, OR = 2.5; rs1828682, located in the intron of *MGST1* gene on chromosome 12, p-value = 4.82E-06, OR = 0.32).

3. *HLA-DPA1*01:03* (Pc = 1.21E-06, OR = 0.54) and *HLA-DPB1*02:01* (Pc = 1.00E-03, OR = 0.5) exhibited significant protective effects in CHB susceptibility. *HLA-DPA1*02:02* (Pc = 6.32E-05, OR = 1.63), *HLA-DPB1*05:01* (Pc = 5.20E-05, OR = 1.72), *HLA-DPB1*13:01* (Pc = 2.16E-02, OR = 1.60), and *HLA-DQB1*03:03* (Pc = 6.47E-04, OR = 1.84) were significantly associated with susceptibility to CHB in the Thai population.
4. HLA haplotypes (two-locus and three-locus) were estimated using Arlequin software and association tests were performed using Pearson's chi-squared test. It was observed that *HLA-DPA1*01:03-HLA-DPB1*02:01* (Pc = 3.39E-04, OR = 0.47) was the only protective haplotype, while *HLA-DPA1*02:02-DPB1*05:01* (Pc = 1.33E-04, OR = 1.68), *HLA-DQB1*03:03-DPB1*05:01* (Pc = 3.30E-03, OR = 2.27), *HLA-DQB1*05:02-DPB1*13:01* (Pc = 9.20E-03, OR = 5.4), *HLA-DQB1*03:03-DPA1*02:02-DPB1*05:01* (Pc = 9.68E-03, OR = 2.7) were found to be significantly associated with susceptibility to HBV persistence.

The second part of this study was the first GWAS for host genetic susceptibility factors for CHB in the Thai population and successfully replicated association of HLA genes and alleles with susceptibility to CHB. This is first time to identify susceptible effect of *HLA-DPB1*13:01* with CHB. In addition, this study also reported a number of novel candidate genes and loci with suggestive evidence of association. This study contributes to a better understanding of host genetic susceptibility factors for the development and pathogenesis of CHB. For these reasons, we consider the candidate worthy of a Ph.D degree.