［課程－２］

審査の結果の要旨

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Idiopathic nephrotic syndrome (INS) is the most common glomerular disease in children, characterized by heavy proteinuria, hypoalbuminemia, edema and dyslipidemia. In Japan, the estimated incidence of INS is 6.49 cases/100,000 children annually. Between 80 and 90% of patients achieve remission with steroid therapy (steroid-sensitive NS [SSNS]). However, the underlying pathogenesis and genetic background of INS is not well understood. Furthermore, there's no published genome-wide association study (GWAS) working on childhood-onset INS in the Japanese population yet. The current study focused on patients with childhood SSNS, aiming to identify the genetic variants contributing to the disease susceptibility. The current study consists of two parts.

The first part was a two-stage GWAS for childhood SSNS in the Japanese population. The results of the first part are summarized as follows:

1. In the discovery stage, 224 patients with childhood SSNS and 419 adult healthy controls were obtained from the Japanese population, and genotyped using Affymetrix 'Japonica Array'. After quality control (QC) procedure, 224 cases and 412 controls with 495,895 autosomal SNPs were retained for association analysis. The most significant association was detected in the *HLA-DR/DQ* region using Cochran-Armitage trend test (rs4642516, $P_{GC\text{-}corrected}=5.44\times10^{-10}$). Then, whole-genome imputation was performed using 2KJPN reference panel, which was generated using whole-genome sequence data of 2,049 healthy Japanese individuals by Tohoku Medical Megabank. 224 cases and 412 controls with 4,105,543 autosomal SNPs and short insertions and deletions (INDELs) passed QC and were included in the association analysis. 10 SNPs and short insertions and deletions (INDELs) were selected from *HLA-DR/DQ* region and another two candidate loci ($P_{GC\text{-}corrected} < 1\times10^{-5}$) for further validation and replication. 5 SNPs were genotyped by DigiTag2 assay successfully in discovery cases as validation (mean concordance 97%).

2. To confirm the associations, candidate variants were genotyped in an independent Japanese sample set including 216 cases and 719 healthy controls by DigiTag2 assay. Two SNPs from *HLA-DR/DQ* region were replicated (rs4642516, 21kb 5'of *HLA-DQB1*, $P=6.69\times10^{-13}$; rs3134996, 705bp 5' of *HLA-DQB1*, $P=9.44\times10^{-10}$) using Cochran-Armitage trend test. Combined analyses were performed using data in discover stage and replication stage. The two SNPs showed significant association under allelic and dominant model [rs4642516, combined $P=7.84\times10^{-23}$, odds ratio (OR)= 0.33 under allelic model, combined $P=4.87\times10^{-23}$, OR= 0.29 under dominant model; rs3134996, combined $P=1.72\times10^{-25}$,

OR=0.29 under allelic model, combined $P=9.57\times10^{-21}$, OR= 0.28 under dominant model].

3. There was no secondary signal in *HLA* region when conditioning on the top SNP in *HLA-DR/DQ* region.

4. 9.7% of variance (Nagelkerke's pseudo-R^2) in Japanese childhood SSNS can be explained by rs4642516 (the top SNP in *HLA* region).

The second part of the study was fine-mapping for the significant association in *HLA* region. HLA-imputation and direct HLA-typing were conducted at 4-digit level. The results of the second part are summarized as follows:

1. Six classical *HLA* genes in both class I and class II regions (*HLA-A*, *-C*, *-B*, *-DRB1*, *-DQB1*, and *-DPB1*) were imputed in the discovery sample set (224 cases and 412 controls) using Japanese-specific reference by 'HIBAG' R package. A total of 197 cases and 411 controls passed post-imputation QC (call-threshold>0.4) and were included in subsequent analyses. Significant *HLA* alleles and haplotypes were identified using imputed data. *HLA-A*02:06* (Pc=8.84×10^{-3}, OR=1.91), *HLA-DRB1*08:02* (Pc=2.56×10^{-4}, OR=2.79), and *HLA-DQB1*03:02* (Pc=4.14×10^{-4}, OR=2.08) were highly associated with the susceptibility to Japanese childhood SSNS. *HLA-A*33:03* (Pc=2.43×10^{-3}, OR=0.30), *HLA-B*44:03* (Pc=1.25×10^{-4}, OR=0.13), *HLA-C*14:03* (Pc=5.25×10^{-5}, OR=0.13), *HLA-DRB1*13:02* (Pc=7.47×10^{-4}, OR=0.18), and *HLA-DQB1*06:04* (Pc=3.40×10^{-5}, OR=0.07) showed significantly protective effects. *HLA-DRB1*08:02-DQB1*03:02* (*Pc*=4.34×10^{-6}, OR=4.33) and *HLA-DRB1*13:02-DQB1*06:04* (*Pc*=6.17×10^{-5}, OR=0.07) demonstrated the most significant positive and negative association, respectively.

2. As validation, HLA-DRB1/-DQB1 genotyping was conducted in discovery sample set using PCR-SSO (sequence-specific oligonucleotide probing) method on Luminex platform. The concordance rate between the imputed (passed post-imputation QC) and genotyped *HLA-DRB1/-DQB1* genotypes was 99.2%.

3. In replication stage, HLA-DRB1/-DQB1 genotyping was conducted successfully in 213 patients and 450 healthy controls. High-accuracy HLA-imputation was conducted in 269 controls using previous GWAS data and 260 controls passed the post-imputation QC. Almost all the significantly associated *HLA-DRB1/-DQB1* alleles and *HLA-DRB1-DQB1* haplotypes were replicated in replication sample set except for *HLA-DQB1*03:02*. Combined analyses were conducted using validation dataset and replication dataset. *HLA-DRB1*08:02* (Pc=1.82×10^{-9}, OR=2.62), and *HLA-DQB1*06:04* (Pc=2.09×10^{-12}, OR=0.10) were considered as primary *HLA* alleles with Japanese childhood SSNS. *HLA-DRB1*08:02-DQB1*03:02* (Pc=7.01×10^{-11}, OR=3.60) was identified as the most significant susceptibility haplotype and showed more significant and stronger association than single *HLA* allele. *HLA-DRB1*13:02-DQB1*06:04* (Pc=4.18×10^{-12}, OR=0.10) was identified as the most significant protective haplotype.

4. Consistent with previous researches in the Japanese population, single-tag SNP rs3129888 works with high sensitivity and specificity for capturing the susceptibility genetic factors *HLA-DRB1*08:02* and *HLA-DRB1*08:02-DQB1*03:02* in this study. Single-tag SNP rs3129888 could be used as an economical biomarker to capture the susceptibility genetic factors in clinical practice.

This study is the first GWAS for childhood SSNS in the Japanese population. Using hypothesis-free method, the predominant role of *HLA-DR/DQ* region in the pathogenesis of childhood SSNS was detected and confirmed in the Japanese population. *HLA* association was dissected by high-resolution HLA fine-mapping. Primary *HLA* alleles and disease-associated *HLA-DRB1-DQB1* haplotypes were identified, providing better understanding of the disease mechanism from molecular and functional aspects. The findings indicate that HLA fine-mapping after SNP-based analysis is essential for a better understanding of disease mechanism. For these reasons, we consider the candidate worthy of a Ph.D. degree.