The clinical trial information provided in this public disclosure synopsis is supplied for informational purposes only.

Please note that the results reported in any single trial may not reflect the overall potential risks or benefits of a product which are based on an evaluation of an entire research program.

Before prescribing any Takeda products, healthcare professionals should consult prescribing information for the product approved in their country.

10.0 CONCLUSIONS

It has been hypothesized that host genetic, immunologic, and environmental factors are important in the pathogenesis of DILI and a growing number of genome-wide association studies (GWAS) able to identify individuals at risk for developing DILI could potentially make otherwise safe and efficacious drugs available for use. [5,11,20]. The GWAS for the specific drugs such as flucloxacillin, amoxicillin clavulanate and lumiracoxib identified the MHC (Major Histocompatibility Complex) signals. The population attributable fraction of HLA-B*5701 is 0.64 when treated with flucloxacillin and Compared to the drug specific risk of DILI, it is difficult to identify common genetic factors for DILI risk due to a variety of drugs [23] even though the GWAS showed the sufficient statistical power (>80%) to detect single-nucleotide polymorphisms (SNPs) conferring relative risk more than two.

We expected that the statistical power of this study could be enough because previous reports showed that the relative risks of the SNPs to DILI due to flucloxacillin and amoxicillin clavulanate were high [4,5]. If the relative risk of this study is same as that in the flucloxacillin case, the statistical power could be more than 0.95 in the case that the MAF is 0.05. However, the odds ratios of SNPs identified in the study are lower than that in the flucloxacillin case. A replication study is required to confirm the association with DILI in the study. It is difficult to collect DNA samples from DILI, because development of TAK-475 was discontinued.

We have conducted two independent GWAS studies with different Caucasian controls, one is iControl from Illumina and the other is from WTCCC and 23 Caucasian TAK-475 DILI cases. The significant SNPs associated in an intron of HDAC9 were observed in both studies. HDAC9 gene encodes an enzyme “histone deacetylase 9”. It’s known the protein encoded by this gene has sequence homology to members of the histone deacetylase family, which alters chromosome structure and affects transcription factor access to DNA. HDAC9 plays key roles in the development and differentiation of several types of cells including T-cells [6,21,26]. HDAC9 deficiency resulted in local accumulation of acetylated H3 at IL-4 promoter. This alternation at molecular level led the mRNA expression of IL-4 in spleen, serum and CD4+ T cells from HDAC9 deficient mice increased dramatically compared with control mice [26]. IL-4 promotes hapten-mediated inflammatory process in the liver and IL-4 in CD4+ T cells induces liver injury by activation of JAK1/STAT6 pathway in hepatocytes [13,9]. In this way, HDAC9 deficiency or decreased expression due to the variation in the HDAC9 region may influence the susceptibility to TAK-475 related liver toxicity. The association of the SNP in an intron of HDAC9 when MEX like subjects were taken out from the cases requires further experiments to confirm its risk to DILI. HDAC inhibitor and HDAC9 knockout mice can be useful for in vitro or in vivo studies to confirm the relationship between TAk-475 induced river injury with the SNP in HDAC9.

The GWAS study with five Japanese TAK-475 DILI cases and JPDSC controls did not find any significant SNPs. It might be due to the less statistic power with only five cases, however, the allele frequency of SNPs, which were associated the DILI of TAK-475 in Caucasian subjects, were major alleles in Japanese population. This result demonstrates that the different risk factors involved the DILI of TAK-475 in Japanese population.

The other SNPs in an intron of SCML4 and METAP1 also show moderate but robust associations with DILI of TAK-475. SCML4 could be a transcription factor but its molecular functions remains unknown. The high mRNA expression of SCML4 in the lymph nodes, spleen and CD8+ T cells may suggest its immune related functions[10]. Although METAP1 is also highly expressed in B cells and T cells in mice spleen, there is limited knowledge about its biological functions [10]. MATAP1 controls not only cell cycle and cell proliferation [4,7] but also may control immune reactions.

The mechanism of DILI of TAK-475 is likely to be different from those of other drug induced liver injuries such as flucloxacillin and amoxicillin clavulanate, because MHC region wasn't identified as the risk factor in our studies. The candidate approach with *ex vivo* and the genotyping of ADME gene with DMET were expected to help us understand DILI mechanism due to TAK-475. The result of DMET might suggest that no any genes or other factors related ADME of TAK-475 was involved in DILI of TAK-475.

Finally, the present study demonstrates the potential of GWAS to discover genes or pathway that potentially mediated the serious adverse events such as DILI during the development by using the existing population control instead of matched control from the clinical studies. Although the further studies using another sets of clinical DNA sample confirm our results, it suggests that HDAC9 might be involved in DILI of TAK-475 but not ADME gene or other factors related TAK-475 metabolism or transports.