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TAP2 drives HLA-B*13:01-linked dapsone hypersensitivity syndrome tolerance and reactivity

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Abbreviations: DHS, dapsone hypersensitivity syndrome; TAP, transporter associated with antigen presentation; APCs, antigen-presenting cells; PPV, positive predictive value; GO, gene ontology; SCARs, severe cutaneous adverse reactions; PBMCs, peripheral blood mononuclear cells; qPCR, quantitative PCR; ELISPOT, enzyme-linked immunospot; CT, cycle threshold; EBV, epstein-Barr virus.

Abstract

Dapsone hypersensitivity syndrome (DHS) is restricted to human leukocyte antigen HLA-B*13:01. However, the positive predictive value for HLA-B*13:01 is only 7.8%. To explore potential coexisting factors involved in the occurrence of DHS, we carried out a genome-wide association study and a genome-wide DNA methylation profile analysis comparing DHS patients with dapsone-tolerant control subjects (all carrying HLA-B*13:01).
No non-HLA SNPs associated with DHS were identified at the genome-wide level. However, the pathway of antigen processing and presentation was enriched in DHS patients and the gene transporter associated with antigen presentation \((TAP2)\) was identified. Expression of \(TAP2\) and its molecular chaperone, \(TAP1\) were validated by qPCR and \textit{in vitro} functional experiments were performed. The results showed DHS patients have higher mRNA levels of \(TAP1\) and \(TAP2\), and an enhanced capacity for antigen-presenting cell (APC) activating dapsone specific T cells compared to dapsone-tolerant controls. Activation of dapsone-specific T cells was inhibited when TAP function of APCs was impaired. This study demonstrates that epigenetic regulation of \(TAP1\) and \(TAP2\) affects the function of APCs and is a critical factor that mediates the development of DHS.

**Keywords:** Dapsone hypersensitivity syndrome, antigen presentation, drug hypersensitivity, T cells, methylation

**Introduction**

Dapsone, also known as diaminodiphenyl sulfone, is an aniline derivative that is used for the treatment of infections and chronic inflammatory diseases. Dapsone hypersensitivity syndrome (DHS), caused by the intake of dapsone, is a life-threatening allergic syndrome characterized by fever, eruptions, lymphopathy, and hepatic function abnormalities (Richardus and Smith, 1989). DHS occurs in approximately 0.5–3.6% of patients treated with dapsone and has a high mortality of 9.9% (Zhang et al., 2013). Previous studies have identified a human leukocyte antigen, \(HLA-B*13:01\), as a major risk factor for DHS, with a high negative predictive value of 99.8% and a low positive predictive value (PPV) of 7.8% (Zhang et al., 2013). Further analysis revealed five amino acid variants (positions 133, 142, -17, 11, and 13) in high-linkage disequilibrium with \(HLA-DRB1\) that conferred susceptibility to this condition. When \(HLA-B*13:01\) and \(DRB1\) were combined as risk predictors, the PPV increased to 9.2% compared to \(HLA-B*13:01\) alone (Yue et al., 2018). However, it remains unclear why a larger
fraction of individuals carrying \textit{HLA-B*13:01} do not develop DHS after administration of dapsone.

It is well established that host genetic predisposition plays a critical role in the development of drug-induced severe cutaneous adverse reactions (SCARs). A series of HLA molecules have been associated with SCARs, including \textit{HLA-B*57:01} for abacavir hypersensitivity (Mallal \textit{et al.}, 2008), \textit{HLA-B*58:01} for allopurinol-SCARs (Hung \textit{et al.}, 2005), and \textit{HLA-B*15:02} for carbamazepine-Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) (Chung \textit{et al.}, 2004). In addition, non-HLA molecules, \textit{CYP2B6} and \textit{CYP2C9}, have been shown to be associated with nevirapine hypersensitivity (Carr \textit{et al.}, 2014; Ciccacci \textit{et al.}, 2013) and phenytoin-induced SCARs (Chung \textit{et al.}, 2014), respectively. Whether other non-HLA molecules are involved in the pathogenesis of DHS requires further investigation. In addition to genetic factors, epigenetic modifications, such as DNA methylation, regulate gene function, with an imbalance of this form of regulation contributing to disease etiology and progression (He \textit{et al.}, 2019). Methylated DNA modifications of the Psoriasis Susceptibility 1 Candidate 1 (PSORS1C1) gene and autophagy-related genes are involved in the pathogenesis of allopurinol-induced SCARs (Cheng \textit{et al.}, 2022) (Sun \textit{et al.}, 2017). A role for epigenetic modifications in the pathogenesis of DHS is unknown.

Thus, to comprehensively investigate factors that mediate the development of DHS, a genome-wide association study (GWAS) and genome-wide DNA methylation profiling were performed in DHS patients and dapsone-tolerant control subjects. All individuals were positive for the \textit{HLA-B*13:01} allele and were receiving dapsone for treatment. \textit{In vitro} functional experiments were performed to elucidate the role of \textit{TAPI} and \textit{TAP2} in the pathogenesis of DHS. \textit{TAPI} and \textit{TAP2} were identified, by this study, as factors involved in DHS.

\textbf{Results}
**Genome-wide association analysis**

Genotyping data for 51 DHS patients and 218 dapsone-tolerant control subjects were initially analyzed. After QC filtering, 258,961 overlapping SNPs were analyzed by genome-wide association analysis. After imputation and quality control (QC), 3,574,594 SNPs outside the MHC region were evaluated by log-additive test. SNPs outside the MHC locus (n = 45) had a suggestive association with DHS (P < 1.0 × 10^{-4}). Among these 45 SNPs, 11 SNPs were previously identified (Zhang et al., 2013). 34 newly identified SNPs were subjected to validation.

A total of 62 DHS patients and 319 dapsone-tolerant control subjects were recruited for subsequent validation analysis. A total of 16 candidate SNPs with P < 1.0 × 10^{-4} were successfully genotyped by the validation study. None of these non-HLA SNPs showed a significant association with DHS by replication analysis (P < 0.05) or by combined analysis at the whole-genome level. Summary statistics for the discovery and replication phases, as well as the combined analysis, are shown in Table S1.

**Global genome-wide DNA methylation profile of DHS**

Genome-wide DNA methylation profiles were compared between 31 DHS patients and 31 dapsone-tolerant control subjects (all carrying HLA-B*13:01) from the discovery set. A total of 759,996 CpG sites were identified by the Infinium Methylation 850k BeadChip, in which 11,181 CpG sites reached epigenome-wide significance (false discovery rate, FDR < 0.05). The distribution of these CpG sites within chromosomes is shown in Figure 1a. Among 11,181 CpG sites, there were 5,566 hypermethylated CpG sites (49.8%) and 5,615 hypomethylated CpG sites (50.2%). Based on the location of methylated regions, these 11,181 CpG sites were classified into six classes: untranslated regions (1,857, 16.6%), transcription start site (2,678, 24.0%), promoter (1,819, 16.3%), enhancer (1,626, 14.5%), gene body (5,493, 49.1%), and unclassified (2,133, 19.1%). The methylation status of the six classes is
shown in Figure 1b.

Because promoter-associated methylation likely affects gene expression, the top 500 significant hypomethylation CpG sites which were annotated as “Promoter_Associated” by the Methylation Consortium were selected from 11,181 CpG sites. The heatmap for the 500 selected CpGs is shown in Figure 1c. To identify immune pathways and key immune-related genes involved in the pathogenesis of DHS, we performed Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis. GO enrichment analysis found 575 biological processes were significantly associated with DHS (P< 0.05), including “GO:0002479:antigen processing and presentation of exogenous peptide antigen via MHC class I, TAP-dependent”, which is a key step for activating T cells. KEGG Pathway enrichment analysis found 24 pathways were significantly associated with DHS (P< 0.05), including “hsa05340:primary immunodeficiency pathway”, which shared the key gene TAP2 (Figure 1d and 1e). Two CpG sites (site 1: cg23560159, Chr6:32805748 and site 2: cg13563634, Chr6:32805684) associated with TAP2 were selected for validation by pyrosequencing. The validation results showed that only Site 1 (cg23560159, Chr6:32805748) of TAP2 was consistent with the discovery set (Figure 2a and 2b).

mRNA expression analysis

To determine whether dysregulated methylation affects gene expression, qPCR was performed to compare gene expression between DHS patients and dapsone-tolerant control subjects. As expected, TAP2 mRNA levels were up-regulated in DHS patients compared to dapsone-tolerant control subjects (Figure 2c). TAP2 is essential for the MHC-I antigen presentation pathway (McCluskey et al., 2004), which in complex with TAP1 mediates unidirectional translocation of endogenous protein antigens into the endoplasmic reticulum. Expression of TAP1 was assessed. Compared to the dapsone-tolerant control subjects, mRNA levels of TAPI were also up-regulated in DHS patients (Figure 2d). Correlation analysis
demonstrated expression of $TAP1$, in peripheral blood mononuclear cells (PBMCs) of 43 donors, was positively related to $TAP2$ (Figure 2e).

**Functional differences in antigen-presenting cells (APCs) between DHS patients and dapsone-tolerant control subjects**

To determine whether dapsone specific T cells exist in the PBMCs of DHS patients, PBMCs from DHS patients and dapsone tolerant control subjects (all carrying $HLA-B^*13:01$) were stimulated with dapsone (100 $\mu$M) for six days and then analyzed by enzyme-linked immunospot (ELISpot). After stimulation with dapsone, responding T cells were specifically activated to produce IFN-γ by PBMCs of DHS patients but not by PBMCs of dapsone tolerant control subjects (Figure 3a). To determine whether CD4+T cells or CD8+T cells responded to dapsone, flow cytometric analysis was performed. After dapsone stimulation, CD8+T cells produced IFN-γ, with only slight CD4+T cell activation (Figure 3b). These results suggest that CD8+T cells and to a lesser extent CD4+T cells act as effector cell populations involved in the pathogenesis of DHS.

To explore functional APC differences between DHS patients and dapsone-tolerant control subjects, EBV-B cell lines were generated to serve as APCs and dapsone-specific T cells were generated to serve as effectors. $HLA-B^*1301$-positive EBV-B cell lines from DHS patients or dapsone-tolerant control subjects were co-cultured with dapsone-specific T cells. Compared to dapsone-tolerant control subjects, dapsone treated EBV-B cell lines from DHS patients activated a greater dapsone-specific T cell response as judged by IFN-γ ELISPOT assay (Figure 3c). $TAP1$ and $TAP2$ mRNA levels of the EBV-B cell lines were assessed by qPCR. The results showed a consistent up-regulated expression of $TAP1$ and $TAP2$ by EBV-B cells from DHS patients compared to dapsone-tolerant control subjects (Figure 3d). These results suggest that functional differences in APCs are critical to the development of DHS, likely due to regulated expression of $TAP2$ and $TAP1$. 
The dapsone-specific T cell response is TAP-dependent

TAP is the member of the superfamily of ATP-binding cassette transporters, that is composed of TAP1 and TAP2 (Dean and Annilo, 2005). The MHC-I antigen presentation pathway is TAP-dependent, with functional loss of TAP resulting in defective peptide loading and reduced surface expression of MHC-I (Chessman et al., 2008; McCluskey et al., 2004). To explore the immune significance of TAP in the activation of dapsone-specific T cells, TAP function was impaired by ICP47 treatment of C1R-HLA-B*13:01 APCs. In this manner, ICP47 was shown to inhibit surface expression of HLA-I molecules on the APCs (Figure 4a). Correlation analysis showed expression of TAP1 and TAP2 to be positively correlated with HLA-B (Figure 4b and 4c). When incubated with TAP impaired C1R-HLA-B*13:01 APCs, dapsone-specific T cells showed a reduced reactivity compared to incubation with C1R-HLA-B*13:01 APCs infected with a negative control lentiviral vector (Figure 4d).

Discussion

The development of pharmacogenomics advanced understanding of genetic predispositions for drug-induced SCARs. Identification of a number of HLA loci associated with drug-induced SCARs has reduced the incidence of disease by successful translation of some into clinical usefulness (Liu et al., 2019). However, most individuals who carry HLA risk loci do not develop adverse reactions even when exposed to the culprit drug, e.g. DHS. Exploring the mechanistic basis for HLA drug-induced SCARs is important for the further development of personalized precision medicine. This study assessed factors that influence DHS and performed functional studies to evaluate the role of TAP1 and TAP2 in the pathogenesis of DHS. The results of this study have the potential to identify the underlying basis for drug-induced SCARs.

In the present study, GO analysis revealed that the antigen processing and presentation related pathway was enriched, which led us to hypothesize that APC function was different
between DHS patients and dapsone-tolerant control subjects. APCs are endowed with antigen sensing, processing, and presentation machinery, allowing for their important role in the activation of drug/antigen-specific T cells (Cardone et al., 2018). Dendritic cells (DCs) perform immune co-stimulatory functions and boost DC maturation, which are needed for abacavir activation of CD8+ T lymphocytes (Cardone et al., 2018). Consistent with these reports, we found that the ability of APCs to activate dapsone specific T cells enhanced in DHS patients compared to dapsone-tolerant controls. These results highlight the importance of APCs in mediating tolerance and reactivity of DHS.

To further investigate the underlying mechanism of functional difference of APCs between DHS patients and dapsone tolerant control subjects, we analyzed differentially methylated genes enriched in immune related pathways. We found that antigen processing and presentation as well as primary immunodeficiency pathways shared the common gene, TAP2. Further analysis found TAP2 to have greater mRNA expression in DHS patients compared to dapsone tolerant control subjects. TAP2 and its molecular chaperone TAP1, are members of the ABC family, located within the MHC class II region between the DPB1 and DQB1 loci (Trowsdale et al., 1990), playing important roles in the function of APCs. Functional loss of TAP results in defective presentation of abacavir to CD8+ T cells in abacavir hypersensitivity (Chessman et al., 2008). Inherited human deficiency in TAP reduces the expression of HLA-I on the cell surface (de la Salle et al., 1994). Consistent with these results, we found HLA-I expression to be lost when TAP function was impaired in the C1R-HLA-B*13:01 cell line and that impairment of TAP function inhibited the response of T cells to dapsone.

Recently, more than 40 genetic risk loci have been associated with drug induced-SCARs (Alfirevic et al., 2012; Chen et al., 2018; Mustafa et al., 2018). Most of these risk loci are located in the HLA region, implying the importance of HLA molecules in SCARs. However, GWAS and high throughput sequencing studies found that non-HLA molecules are also
involved in the pathogenesis of HLA-linked drug induced-SCARs. For example, both \( HLA-B^{*}15:02 \) and \( CYP2C9^{*}3 \) are associated with phenytoin-related SCARs (Chung et al., 2014). To investigate genetic factors associated with DHS, we carried out a GWAS study and identified no non-HLA risk loci significantly associated with DHS. Furthermore, our previous prospective cohort study showed that 1,251 HLA-B*13:01 negative patients who received dapsone did not develop DHS (Liu et al., 2019). These results suggest that DHS is likely best defined as a single-gene genetic disease. However, more studies with larger numbers of patients need to confirm these observations.

In summary, this study demonstrated DNA methylation status to be associated with \( TAP2 \) and mRNA expression levels of \( TAP2 \) and \( TAP1 \). Further, differences in these characteristics existed between DHS patients and dapsone-tolerant control subjects. The \textit{in vitro} functional analyses and co-culture experiments showed that TAP1 and TAP2 were necessary for APCs to trigger the response of CD8+ T cells to dapsone. Given the unique function of TAP1 and TAP2, this study provides a path by which to explore the basis for other HLA-linked drug reactions.

**Material and Methods**

**Patients and samples**

The GWAS discovery cohort included 39 DHS patients and 88 dapsone-tolerant control subjects from a previously published GWAS dataset (Zhang et al., 2013). In addition, a newly-added discovery analysis dataset (12 DHS patients and 130 dapsone-tolerant control subjects) was evaluated with Illumina Omni Zhonghua chips (California, USA). An additional 62 DHS patients and 319 dapsone-tolerant control subjects were recruited as a replication cohort. The clinical cohort characteristics of the GWAS evaluated individuals are provided in Table S2. The discovery cohort for the genome-wide DNA methylation profiling included 31 DHS patients and 31 dapsone-tolerant control subjects. The validation set included 55 DHS...
patients and 94 dapsone-tolerant control subjects. Diagnosis of DHS patients was based on the criteria proposed by Richardus and Smith (Richardus and Smith, 1989). All of the participants were leprosy patients treated with dapsone and were HLA-B*13:01-positive. The clinical cohort characteristics of the genome-wide DNA methylation profiling are provided in Table 1. Clinical samples, including DNA and PBMCs, were collected during the DHS recovery disease state. The written informed consent was obtained from each participant. Institutional ethics approvals were reviewed and approved by the ethics committee of the Shandong Provincial Institute of Dermatology and Venereology, Shandong Academy of Medical Science (approval 20190314KYKT004).

**Genome-wide association study**

A two-stage GWAS of DHS specimens was conducted. SNPs and sample quality control (QC) criteria were the same as those for the previously published GWAS dataset (Zhang et al., 2013).

Phasing was performed using SHAPEIT version 2. SNP imputation was carried out with IMPUTE version 2.2.2 and the 1,000 Genomes Project Phase I reference panel (March 2012 release) in NCBI Build 37 (hg19) coordinates. The SNPs that could be used with high confidence (info score > 0.8), SNPs with minor allele frequency (MAF) ≥ 5%, SNPs without significant deviation from Hardy-Weinberg equilibrium for controls (P > 1 × 10^{-5}), and SNPs outside the major histocompatibility complex (MHC) region were subsequently analyzed.

The loci that were not replicated in the previously published GWAS were selected for the validation analysis. The SNPs were genotyped using the Sequenom MassARRAY platform. The QC in the replication stage was the same as that in the previously published GWAS dataset (Zhang et al., 2013). The discovery and replication samples were treated as independent samples in the combined analysis.

After using imputation and QC filtering, a log-additive test was performed on the
selected SNPs using SNPTEST version 2.4.1 software. Log-additive SNP association testing in the replication stage and combined analysis were performed using PLINK v1.07.

**DNA methylation chip analysis**

The genomic DNA of peripheral blood was isolated using a DNA extraction kit (QuickGene DNA whole blood kit; Wako Chemicals). DNA methylation sequencing in the discovery set was performed with Infinium Methylation 850k BeadChip (Illumina, San Diego, CA, USA) and validated by pyrosequencing at Genergy Biotechnology (Shanghai, China).

Raw data were filtered and analyzed using the R package “Chip Analysis of Methylation Pipeline” (Tian et al., 2017). The following CpG sites were excluded from analysis: (1) CpG sites that were not detected in at least 5% of samples at a P-value of 0.01; (2) CpG sites that were represented fewer than three times in 5% of samples; (3) random SNPs and non-CpG probes; (4) color channel-switching SNP probes (probes with SNPs as identified in Zhou's Nucleic Acids Research Paper in 2016 (Zhou et al., 2017)); (5) probes that mapped to multiple locations; and (6) CpG sites on X and Y chromosomes. These procedures resulted in 62 samples and 759,888 CpG sites for downstream analyses. Next, β values were normalized using Beta MIxture Quantile dilation and ComBat method to correct for type 1 and 2 probe bias and batch effect, respectively (Johnson et al., 2007). Batch-corrected β values were used to identify differential probes using a linear model in the “limma” R package.

The top 500 differentially hypomethylated sites annotated as “Promoter_Associated” by Methylation Consortium CpG sites were selected, since promoter region is closely related to the regulation of gene expression. To analyze the biological functions corresponding to these hypomethylated sites associated genes, GO and KEGG pathway enrichment analysis were performed by https://www.bioinformatics.com.cn, a free online platform for data analysis and visualization (Luo and Brouwer, 2013; Yu et al., 2012). Among the enriched significant biological process and pathways, we focused on the immune related biological process and
pathways, given the immune nature of DHS. And the shared functional genes were selected and associated CpG sites were validated by pyrosequencing assay (specimens from 55 DHS subjects and 94 dapsone-tolerant control subjects) conducted by Genergy Biotechnology (Shanghai, China). Two CpG site probes of TAP2 were designed and validated successfully. CpG sites were declared significant at the validation stage if the nominal P-value was < 0.025 (0.05/2), corresponding to a Bonferroni-corrected significance for two CpG sites.

**qPCR analysis**

*TAP2* and its molecular chaperone *TAP1* mRNAs were identified by qPCR of genes enriched in the primary immunodeficiency pathway and the antigen processing and presentation of exogenous peptide antigen via MHC class I. The qPCR results were expressed as relative mRNA levels based on the cycle threshold (CT) value.

**RNA sequencing**

Total RNA was extracted from PBMCs of 43 donors with TRIzol reagent (Invitrogen, Carlsbad, CA), with mRNA isolated using oligo(dT) coupled to magnetic beads. cDNA libraries were generated by reverse transcription-PCR, end repair, dA-tailing, adaptor ligation, and PCR amplification. Finally, libraries were constructed with an Illumina Hiseq platform (Illumina).

**Cells and culture**

PBMCs were isolated from fresh blood using Ficoll gradient separation and cryopreserved using a Cryostar™ Freezing Kit (U-CyTech, Netherlands). PBMCs were cultured in medium containing 10% fetal bovine serum (Gibco, USA), 1% penicillin and streptomycin (Gibco), 25 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer (Gibco), 2 mM L-glutamine (Gibco), and 25 µg/mL transferrin (Solarbio, Beijing, China) in Roswell Park Memorial Institute 1640 medium (Gibco).

Stable C1R-HLA-B*13:01 APCs were kindly provided by Dr. Dean Naisbitt from the
University of Liverpool, Department of Pharmacology.

**Generation of EBV-transformed B cell lines**

PBMCs were incubated with supernatant from Epstein-Barr virus (EBV)-producing cell line B95.8 and 0.5 µg/mL cyclosporine A (MCE, New Jersey, USA) overnight at 37°C and 5% CO₂. Then, PBMCs were re-suspended in medium containing 0.5 µg/mL cyclosporine A and cultured for 21 days. The transformed B cell lines were expanded in medium without cyclosporine A.

**T cell isolation**

PBMCs from DHS patients were cultured with dapsone (100 µM) for 14 days and then stimulated with ImmunoCult™ Human CD3/CD28/CD2 T cell activator (STEMCELL Technologies, Canada) in medium supplemented with 10 ng/mL interleukin-2 (PEPROTECH, New Jersey, USA) to expand dapsone-specific T cells. T cells were isolated using positive selection with CD3 microbeads (Miltenyi Biotec, Germany).

**T cell and APC co-culture**

2.5 × 10⁴ EBV-B cells or 1 × 10⁵ C1R-HLA-B*13:01 APCs in 50 µL of medium were co-cultured with 1 × 10⁶ expanded T cells in 50 µL of medium per well in 96-well ELISPOT plates (Dakewe Biotech Co., Ltd., Shenzhen, China). Dapsone was added to the cultures at final concentrations of 25 µM, 50 µM, 100 µM, 200 µM, and 400 µM and cultured for two days. IFN-γ was detected by ELISPOT assay according to the manufacturer’s instructions (Mabtech, Nacka Strand, Sweden).

**Inhibition of TAP1 and TAP2 by ICP47**

The herpes simplex TAP inhibitor, ICP47, was transduced into stable C1R-HLA-B*13:01 APCs via a lentiviral vector at a multiplicity of infection of 100 and selection with hygromycin B at a concentration of 300 µg/mL.

**Flow cytometry assays**
C1R-HLA-B*13:01 APCs and TAP impaired stable C1R-HLA-B*13:01 APCs were surface stained for HLA-I (PE anti-human HLA-A,B,C, Biolegend, USA). Dapsone-specific T cells were stimulated with EBV-B HLA-B*1301-positive cell lines in the presence or absence of dapsone (100 µM) for two days. Cells were surface stained for CD4 (PerCP Anti-human CD4 Antibody, Biolegend, USA) and CD8 (BD Pharmingen™ PE-Cy™7 Mouse Anti-Human CD8, BD, USA), permeabilized, and stained intracellularly for IFN-γ (PE/Dazzle™ 594 IFN-γ, Biolegend, USA). Cells were collected and analyzed by flow cytometry.

Data availability statement

All data generated or used during the study appear in the submitted article.

Conflicts of Interest: The authors state no conflict of interest.

Acknowledgements

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Author contributions

Reference


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*Other Minorities included bai, Buyi, Dai, Dong, Miao, Tujia, Yao, Yi, Chuang
Figure legends

Figure 1. Overview of genome-wide DNA methylation profiling of dapsone hypersensitivity syndrome. (a) Differentially methylated sites in human chromosomes. Manhattan plot of 11,181 CpG sites that reached epigenome-wide significance (false discovery rate, FDR < 0.05). (b) Number of hyper- and hypo-methylation sites in different regions. (c) Heatmap of 500 selected significant CpGs. The KEGG pathway enrichment analysis (d) and Sankey dot GO enrichment analysis (e) of the genes selected from the top 500 significant hypomethylation CpGs sites.

Figure 2. Corresponding up-regulated mRNA expression. DNA methylation level of site 1 (cg23560159) in discovery stage (a) and validation stage (b). The mRNA levels of TAP2 (c) and TAPI (d) of DHS patients compared to dapsone-tolerant control subjects. (e) Correlation analysis showed that the expression of TAPI and TAP2 in PBMCs of 43 donors was positively related.

Figure 3. Functional differences between APCs of DHS patients and dapsone-tolerant control subjects. (a) The PBMCs from DHS patients and dapsone tolerant control subjects were cultured with dapsone (100 μM) for six days and INF-γ release measured by ELISPOT. 2.5 μg/mL PHA served as positive control. DDS, dapsone. (b) Flow cytometry histograms of CD4+T cells and CD8+T cells with intracellular staining for IFN-γ after dapsone stimulation. (c) EBV-B HLA-B*1301-positive cell lines from DHS patients or dapsone-tolerant control subjects were co-cultured at a ratio of 40:1 with dapsone-specific T cells in the presence of dapsone for two days, IFN-γ secretion was detected by ELISPOT. (d) mRNA expression levels for TAPI and TAP2 were significantly higher in EBV-B cells of DHS patients compared to those in dapsone-tolerant control subjects.
Figure 4. Dapsone-specific T cell response was TAP1- and TAP2-dependent. (a) Stable C1R-HLA-B*13:01 APCs were infected with a lentiviral vector encoding herpes simplex TAP inhibitor ICP47. HLA-I expression was reduced compared to stable C1R-HLA-B*13:01 APCs infected with negative control lentiviral vector. Correlation analysis showed that the expression of TAP1 (b) and TAP2 (c) in PBMCs was positively related to HLA-B. (d) IFN-γ secretion representing activation of dapsone-specific T cells was detected by ELISPOT. When the function of TAP1 and TAP2 was impaired by co-expression of the TAP inhibitor ICP47, activation of dapsone-specific T cells by dapsone was reduced.
a) DHS patient and Tolerant

Blank

PHA

DDS (100uM)

b) Without dapsone

With dapsone

CD4

IFN-γ

CD8

T cell maker

Cytokines

T + DHS01-B

T + DHS02-B

T + Tolerant01-B

T + Tolerant02-B

Blank

DDS (25uM)
a) ICP47 vs. NC

b) Scatter plot of HLA-I vs. HLA-B with linear regression line. Correlation coefficient: R = 0.36, p = 0.013

c) Scatter plot of HLA-I vs. HLA-B with linear regression line. Correlation coefficient: R = 0.35, p = 0.021

d) Colony assay images for ICP47 and NC under different conditions:
- Blank
- PHA
- DDS (400uM)
Supplementary Table 1. The 16 successfully validated non-HLA SNPs in discovery stage and validation stage.

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‡ Minor allele/major allele; OR, odds ratio is with respect to the minor allele; F_A, minor allele frequency in DHS; F_U, minor allele frequency in dapsone-tolerant controls; (f) indicates results from fixed-effects meta analysis; NA, not applicable; Phet, P value of heterogeneity