Genetic Variants Associated With Phenytoin-Related Severe Cutaneous Adverse Reactions

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IMPORTANCE The antiepileptic drug phenytoin can cause cutaneous adverse reactions, ranging from maculopapular exanthema to severe cutaneous adverse reactions, which include drug reactions with eosinophilia and systemic symptoms, Stevens-Johnson syndrome, and toxic epidermal necrolysis. The pharmacogenomic basis of phenytoin-related severe cutaneous adverse reactions remains unknown.

OBJECTIVE To investigate the genetic factors associated with phenytoin-related severe cutaneous adverse reactions.

DESIGN, SETTING, AND PARTICIPANTS Case-control study conducted in 2002-2014 among 105 cases with phenytoin-related severe cutaneous adverse reactions (n=61 Stevens-Johnson syndrome/toxic epidermal necrolysis and n=44 drug reactions with eosinophilia and systemic symptoms), 78 cases with maculopapular exanthema, 130 phenytoin-tolerant control participants, and 3655 population controls from Taiwan, Japan, and Malaysia. A genome-wide association study (GWAS), direct sequencing of the associated loci, and replication analysis were conducted using the samples from Taiwan. The initial GWAS included samples of 60 cases with phenytoin-related severe cutaneous adverse reactions and 412 population controls from Taiwan. The results were validated in (1) 30 cases with severe cutaneous adverse reactions and 130 phenytoin-tolerant controls from Taiwan, (2) 9 patients with Stevens-Johnson syndrome/toxic epidermal necrolysis and 2869 population controls from Japan, and (3) 6 cases and 374 population controls from Malaysia.

MAIN OUTCOMES AND MEASURES Specific genetic factors associated with phenytoin-related severe cutaneous adverse reactions.

RESULTS The GWAS discovered a cluster of 16 single-nucleotide polymorphisms in CYP2C genes at 10q23.3 that reached genome-wide significance. Direct sequencing of CYP2C identified missense variant rs1057910 (CYP2C9*3) that showed significant association with phenytoin-related severe cutaneous adverse reactions (odds ratio, 12; 95% CI, 6.6-20; P=1.1×10^-17). The statistically significant association between CYP2C9*3 and phenytoin-related severe cutaneous adverse reactions was observed in additional samples from Taiwan, Japan, and Malaysia. A meta-analysis using the data from the 3 populations showed an overall odds ratio of 1.1 (95% CI, 6.2-18; z=8.58, P<.00001) for CYP2C9*3 association with phenytoin-related severe cutaneous adverse reactions. Delayed clearance of plasma phenytoin was detected in patients with severe cutaneous adverse reactions, especially CYP2C9*3 carriers, providing a functional link of the associated variants to the disease.

CONCLUSIONS AND RELEVANCE This study identified CYP2C variants, including CYP2C9*3, known to reduce drug clearance, as important genetic factors associated with phenytoin-related severe cutaneous adverse reactions.


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Phenytoin (diphenylhydantoin) is a widely prescribed anti-epileptic drug and remains the most frequently used first-line antiepileptic drug in hospitalized patients. Although effective for treating neurological diseases, phenytoin can cause cutaneous adverse reactions ranging from mild rash (maculopapular exanthema) to life-threatening severe cutaneous adverse reactions.\(^3\)\(^-\)\(^6\) Phenytoin-related severe cutaneous adverse reactions include drug reactions with eosinophilia and systemic symptoms (DRESS), Stevens-Johnson syndrome (SJS), and toxic epidermal necrolysis (TEN).\(^4\)\(^-\)\(^6\) Stevens-Johnson syndrome and TEN are characterized by a rapidly developing blistering exanthema of purpuric macules and target-like lesions accompanied by mucosal involvement and skin detachment. Stevens-Johnson syndrome was defined as skin detachment less than 10% of the body surface area, SJS-TEN overlap as skin detachment from 10% to 29%, and TEN as skin detachment greater than 30%.\(^4\)\(^-\)\(^5\) The criteria and scoring system of DRESS include cutaneous involvement with typical rash (eg, exfoliative dermatitis, diffuse maculopapular exanthema), fever, eosinophilia, lymph node enlargement, atypical lymphocytes, internal organ involvement (liver, kidney, central nervous system, lung, heart, muscle), and time of resolution.\(^7\) The maculopapular exanthema phenotype is characterized by generalized cutaneous erythematous macules and papules and is self-limited without systemic involvement. We used 2 methods, the Naranjo score\(^14\) and ALDEN (algorithm of drug causality for epidermal necrolysis),\(^15\) to determine the drug causality as phenytoin. Drug-tolerant patients who had received phenytoin for more than 3 months without evidence of adverse reactions were enrolled as controls from the departments of neurology or neurosurgery of the CGMH health system in Taiwan in 2002-2014. Written informed consent was obtained from each participant. This study was approved by the institutional review board of the ethical standards committee of each study site/institute.

GWAS, Direct Sequencing, and Linkage Disequilibrium Analysis

GWAS was performed using the Affymetrix SNP Array 6.0 platform, which is composed of 909,622 single-nucleotide polymorphisms (SNPs). The genotype calls were generated using the Birdseed method (Birdseed version 2) with Affymetrix Power Tools (version apt-1.10.2). The mean call rate of each array is 98.7% (SD, 0.95%). We excluded SNPs with a call rate of less than 0.90 and \(P<5.5\times10^{-8}\) (0.05/909,622) (\(P<.05\) with Bonferroni correction for multiple comparisons) in a Hardy-Weinberg equilibrium test of data from participants from the general population of Taiwan. We performed GWAS analysis and principal component analysis and constructed a quantile-quantile plot using MATLAB version 8.1 and Bioinformatics Toolbox version 4.3 (MathWorks). After quality control measures and principal component analysis implementation, a total of 854,035 SNPs were used in the GWAS discovery. To investigate functional SNPs, we designed polymerase chain reaction primers (listed in eTable 1 in the Supplement) for direct sequencing (Sanger method) of the exons of associated genes in severe cutaneous adverse reactions cases. Then, the genotypes of missense/nonsense SNPs identified from direct sequencing were further examined in the samples of severe cutaneous adverse reactions cases, phenytoin-tolerant controls, and population controls by TaqMan assays (Life Technologies). Haploview software (version 4.1) was used to draw the linkage disequilibrium maps of chromosome 10: 96.0-97.5 Mb.
Phenytoin-Related Severe Cutaneous Adverse Reactions

Original Investigation Research

Results

For the initial GWAS, direct sequencing of the associated loci, and replication analysis, we enrolled a total of 168 cases with phenytoin-related cutaneous reactions (n=90 severe cutaneous adverse reactions [n=48 SJS-TEN and n=42 DRESS] and n=78 maculopapular exanthemas) and 130 tolerant controls from Taiwan (Table 1). Of the 90 cases with severe cutaneous adverse reactions, 13 patients died as a result of the episode (Table 1). The average daily dose of phenytoin showed no significant difference between the 90 severe cutaneous adverse reactions cases (mean, 314 mg/d; 95% CI, 292-330 mg/d) and 130 phenytoin-tolerant controls (mean, 323 mg/d; 95% CI, 309-337 mg/d; P=.42) (Table 1). Based on the data from the Taiwan National Health Insurance and CGMH databases, the estimated prevalence was 0.24% for phenytoin-related SJS-TEN, 0.21% for phenytoin-related DRESS, and 3.6% for phenytoin-related maculopapular exanthema in Taiwan.

As control participants in the GWAS, we randomly selected 412 healthy individuals from a Taiwan biobank under a nationwide population study, which comprises 9980 Han Chinese descendants.16 There was no self-report of adverse drug events by any of these 412 participants from Taiwan, where 98% of the population is made up of Han Chinese. We performed the GWAS using samples from 60 cases of phenytoin-related severe cutaneous adverse reactions (n=38 SJS-TEN and n=22 DRESS) initially enrolled from a referral center (CGMH) and the 412 controls from Taiwan. The principal component analysis plots (eFigure 1 in the Supplement) could not separate the 60 severe cutaneous adverse reactions cases from 412 general controls, suggesting that there is no population stratification between cases and controls. The principal component analysis located most of the Taiwanese severe cutaneous adverse reactions cases as among southern, central, and northern Han Chinese of mainland China (eFigure 2 in the Supplement).

The GWAS discovered a cluster of 16 SNPs on chromosome 10q23.33 (96.4-97.0 Mb) that reached the genome-wide significance threshold (P < 5 × 10−8) for association with phenytoin-related severe cutaneous adverse reactions (Figure 1). Eight SNPs with the lowest P values were located on CYP2C genes, comprising CYP2C18 (NCBI Entrez gene 1562), CYP2C9 (NCBI Entrez gene 1557), CYP2C9 (NCBI Entrez gene 1559), and CYP2C8 (NCBI Entrez gene 1558) (Table 2). The quantile-quantile plot confirmed a marked excess of significantly associated SNPs on chromosome 10 (eFigure 3 in the Supplement). Direct sequencing of the CYP2C genes of patients identified 2 missense variants, rs1057910 (CYP2C9*3; p.I359L) and rs3758581 (CYP2C9*1C; p.V331I), showing statistically significant association with phenytoin-related severe cutaneous adverse reactions (Table 2 and containing the CYP2C region. We calculated the D′ and r2 values to estimate the independence of the SNPs in the samples.

Analysis of Concentrations of Plasma Phenytoin

We obtained convenience plasma samples from phenytoin-tolerant controls (including those with continuous use of phenytoin) and those who were able to discontinue phenytoin therapy and provided their serial blood samples before or after drug withdrawal) and severe cutaneous adverse reactions cases. Plasma samples of controls who received the maintenance dosage were collected within 24 hours after the last dose of phenytoin. Available samples from phenytoin-tolerant controls and patients with severe cutaneous adverse reactions were obtained before or after withdrawal of phenytoin. The date of drug withdrawal in patients with severe cutaneous adverse reactions was usually the same day or near the onset of severe cutaneous adverse reactions when phenytoin was recognized as the associated drug. The plasma concentration of total phenytoin in samples was determined by fluorescence polarization immunoassay using AssyM Phenytoin Assay (Abbott) in the Department of Laboratory Medicine of the CGMH (College of American Pathologists number 3291201-02). Standard calibrators (0.0, 2.5, 5.0, 10.0, 20.0, and 40.0 μg/mL) were used to generate the standard curve. The assay system has a sensitivity of 0.5 μg/mL. This sensitivity is defined as the lowest measurable concentration that can be distinguished from zero with 95% confidence. Interday and intraday variability in precision were determined using human serum with 6.9, 14.0, and 24.0 μg/mL of phenytoin added, which yielded a coefficient of variation of less than 2.9%. Accuracy by recovery was determined by adding phenytoin to human serum and to buffer at concentrations of 2.5, 4.0, 8.0, 12.0, 16.0, 20.0, 30.0, and 36.0 μg/mL, and the mean recovery was 101.5% (SD, 3.9%).

Statistical Analysis

We conducted the statistical analysis for the association by comparing the allele or genotype frequencies between cases and controls in modes of inheritance (additive model, recessive model, or dominant models). The associations were examined by Fisher exact tests and rank-ordered according to the lowest P value in these models. All P values were 2-tailed. A Bonferroni correction was applied for the multiple comparisons and adjusted the P values using the numbers of tests (n=854 035 SNPs for GWAS, n=17 for HLA-A genotypes, and n=36 for HLA-B genotypes). A corrected P<.05 was considered to be statistically significant, and significant P values were P<.0029 for HLA-A (0.05/17), P<.0014 for HLA-B (0.05/36), and P<5.9 × 10−8 for GWAS (0.05/854 035). Odds ratios (ORs) were calculated using a Haldane modification, which added 0.5 to all cells to accommodate possible zero counts. Fisher exact tests, Bonferroni correction, and OR calculation were performed by MATLAB version 8.1 and Statistics Toolbox version 8.2 (MathWorks), and a meta-analysis was conducted using Review Manager (RevMan) version 5.2. Pooled ORs using a random-effects model were calculated from studies with phenytoin-related severe cutaneous adverse reactions or population controls and CYP2C9*3 allele analysis. Study heterogeneity was investigated by calculating r2 and P. The statistical significance was defined as P < .05. The concentrations of plasma phenytoin in the different groups were compared by nonparametric tests.

Additional information regarding methods to determine drug causality, estimates of the prevalence of phenytoin-related cutaneous adverse reactions, and HLA genotyping methods is provided in the eAppendix in the Supplement.
The association between the 10 variants and phenytoin-related severe cutaneous adverse reactions was replicated in an independent set of 30 cases of phenytoin-related severe cutaneous adverse reaction (n=10 SJS-TEN and n=20 DRESS) recruited from the Taiwan Severe Cutaneous Adverse Reactions Consortium and 130 phenytoin-tolerant controls (Table 2). All 10 SNPs in the 412 general controls were in Hardy-Weinberg equilibrium (Table 2). The 10 SNPs are common (minor allele frequencies ≥0.19) in severe cutaneous adverse reactions cases, yet rare (minor allele frequencies 0.017-0.063) in the population controls from Taiwan (n = 412) and the southern (n = 500), central (n = 500), and northern (n = 500) Han Chinese samples (eTable 3 in the Supplement). The 10 SNPs showed strong linkage disequilibrium in the data sets of 412 controls and 90 severe cutaneous adverse reactions cases but a smaller linkage disequilibrium block in 130 phenytoin-tolerant controls (Figure 2 and eTables 4-6 and eFigure 4 in the Supplement). Among the 7 haplotypes inferred from 3 SNPs (rs3758581, rs1057910, and rs6583967), the risk haplotype (haplotype 2) was absent in 130 phenytoin-tolerant controls and showed significant association with phenytoin-related severe cutaneous adverse reactions (eFigure 5 in the Supplement). Because the estimated prevalence of phenytoin-related severe cutaneous adverse reactions is very low in Taiwan, the genotyping data of 130 phenytoin-tolerant controls and 412 population controls was combined. All 10 SNPs exhibited significant association with phenytoin-related severe cutaneous adverse reactions in the combined-samples analysis (90 cases and 542 controls) and showed P > .05 for heterogeneity between studies (Table 2). Data from the GWAS, replication, and combined-samples analysis all revealed that CYP2C9*3 showed significant association with
phenytoin-related severe cutaneous adverse reactions (OR, 12; 95% CI, 6.6-20; \( P = 1.1 \times 10^{-17} \) in the combined-samples analysis) (Table 2).

We compared the SNP data of the subgroups of 168 patients with phenytoin-related cutaneous adverse reactions and 130 phenytoin-tolerant controls and found that CYP2C9*3 exhibited significant association with phenytoin-related SJS-TEN (OR, 30; 95% CI, 8.4-109; \( P = 1.2 \times 10^{-10} \)), DRESS (OR, 19; 95% CI, 5.1-71; \( P = 7.0 \times 10^{-7} \)), and maculopapular exanthema (OR, 5.5; 95% CI, 1.5-21; \( P = .01 \)) (eTable 7 in the Supplement).

The significant association between CYP2C9*3 and phenytoin-related cutaneous adverse reactions was also noted when comparing data from cases with that of the 412 population controls (eTable 7).

We examined the association between CYP2C9*3 and phenytoin-related severe cutaneous adverse reactions using

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**Figure 1. Genome-Wide Association Scan and Linkage Disequilibrium Map for the CYP2C Region Associated With Phenytoin-Related Severe Cutaneous Adverse Reactions**

![Genome-Wide Association Scan and Linkage Disequilibrium Map](image)

A, Manhattan plot showing associations between CYP2C single-nucleotide polymorphisms (SNPs) and phenytoin-related severe cutaneous adverse reactions. Each dot represents a −\( \log_{10} P \) value calculated by Fisher exact test for the allele frequency in 60 severe cutaneous adverse reaction cases and 412 population controls. The red horizontal line represents \( P = 5.9 \times 10^{-6} \), indicating \( P = .05 \) by Bonferroni correction for the multiple comparisons (0.05/854 035). B, The −\( \log_{10} P \) values of SNPs on the chromosome 10q23.33 (physical position: 96.0-97.5 Mb) and the linkage disequilibrium heat map based on pairwise D’ values from genome-wide association study data from 412 controls. The genomic coordinates are based on the NCBI Human Genome build 37.5, and the standard ideogram of chromosome 10 was taken from the NCBI Human Genome resource site. Top, Single-nucleotide polymorphisms with \( P < .01 \) are indicated by blue and \( P > .01 \) by cyan. The physical position between chromosome 10 (96.4-97.0 Mb), which spans CYP2C18, CYP2C19, CYP2C9, CYP2CB, and C10orf29 genes, is indicated by pink lines. Bottom, Black triangles mark the linkage disequilibrium blocks. A D’ value of 1 indicates that the examined loci exhibit complete dependency while a value of 0 demonstrates the independence of one another. The colors represent the D’ values: red (0.5 ≤ D’ values ≤ 1), white to pink (0>D’ values >0.5), purple (D’ = 0).
Table 2. Ten Significant SNPs Associated With Phenytoin-Related Severe Cutaneous Adverse Reactions in the GWAS Discovery, Direct Sequencing, Replication, and Combined Samples

<table>
<thead>
<tr>
<th>SNP</th>
<th>Position on Chromosome 10 (bp)*</th>
<th>Nearby Gene (Location)</th>
<th>Minor allele</th>
<th>GWAS Discoveryb</th>
<th>Replication Analysisc</th>
<th>Combinationd</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>MAF Cases</td>
<td>Controls</td>
<td>OR (95% CI)</td>
<td>MAF Cases</td>
</tr>
<tr>
<td>rs17110192</td>
<td>10q23.33 (96441927)</td>
<td>CYP2C18 (5′UTR)</td>
<td>C</td>
<td>0.2</td>
<td>0.026</td>
<td>1.5×10⁻¹¹</td>
</tr>
<tr>
<td>rs3758851</td>
<td>10q23.33 (9662623)</td>
<td>CYP2C19 (exon 7)</td>
<td>A</td>
<td>0.21</td>
<td>0.03</td>
<td>3.4×10⁻¹¹</td>
</tr>
<tr>
<td>rs1710321</td>
<td>10q23.33 (9639896)</td>
<td>CYP2C9, CYP2C19 (exon 7)</td>
<td>G</td>
<td>0.2</td>
<td>0.026</td>
<td>1.5×10⁻¹¹</td>
</tr>
<tr>
<td>rs9332093</td>
<td>10q23.33 (9669555)</td>
<td>CYP2C9 (5′UTR)</td>
<td>G</td>
<td>0.2</td>
<td>0.026</td>
<td>1.5×10⁻¹¹</td>
</tr>
<tr>
<td>rs1057910</td>
<td>10q23.33 (9674103)</td>
<td>CYP2C9 (exon 7)</td>
<td>C</td>
<td>0.21</td>
<td>0.024</td>
<td>1.5×10⁻¹²</td>
</tr>
<tr>
<td>rs9332245</td>
<td>10q23.33 (96749181)</td>
<td>CYP2C9 (3′UTR)</td>
<td>A</td>
<td>0.2</td>
<td>0.026</td>
<td>1.5×10⁻¹¹</td>
</tr>
<tr>
<td>rs1592037</td>
<td>10q23.33 (9762328)</td>
<td>CYP2C8 (3′UTR)</td>
<td>A</td>
<td>0.21</td>
<td>0.033</td>
<td>1.0×10⁻¹⁰</td>
</tr>
<tr>
<td>rs6583967</td>
<td>10q23.33 (9814475)</td>
<td>CYP2C8 (Intron)</td>
<td>C</td>
<td>0.21</td>
<td>0.033</td>
<td>1.0×10⁻¹⁰</td>
</tr>
<tr>
<td>rs10882551</td>
<td>10q23.33 (9809573)</td>
<td>CYP2C8 (3′UTR)</td>
<td>T</td>
<td>0.2</td>
<td>0.032</td>
<td>4.6×10⁻¹⁰</td>
</tr>
<tr>
<td>rs1226878</td>
<td>10q23.33 (9671504)</td>
<td>C10orf129 (Intron)</td>
<td>C</td>
<td>0.2</td>
<td>0.03</td>
<td>1.6×10⁻¹⁰</td>
</tr>
</tbody>
</table>

Abbreviations: GWAS, genome-wide association study; HET, P value of the heterogeneity test between studies; HWE, Hardy-Weinberg equilibrium P values for 412 controls from the general population; MAF, minor allele frequency; OR, odds ratio; SNP, single-nucleotide polymorphism; UTR, untranslated region.


Although no SNPs on HLA region reached genome-wide significance, we examined the HLA association because of the immunological characteristics of phenytoin-related severe cutaneous adverse reactions.3,6,18 Phenytoin-related severe cutaneous adverse reactions showed no link with HLA-A and a very weak association with HLA-B*13:01, HLA-B*15:02, and HLA-B*51:01, in which their P values become nonsignificant after Bonferroni correction (eTable 10 in the Supplement). In the subgroup analysis, only phenytoin-related SJS-TEN showed significant association with HLA-B*15:02 (OR, 5.0; 95% CI, 2.0-13; P = 7.0 × 10⁻⁴; P = .025 after Bonferroni correction) (eTable 10). Adding HLA-B*1502 to CYP2C9*3 genetic screening improved the sensitivity to 62.5% for phenytoin-related SJS-TEN but decreased the specificity (eTable 11 in the Supplement).

The concentrations of plasma phenytoin were determined in the samples of participants, including (1) 90 phenytoin-tolerant controls with continuous use of phenytoin; (2) 11 phenytoin-tolerant controls who were able to discontinue phenytoin therapy; (3) 14 patients with SJS-TEN; and (4) 26 patients with DRESS (Table 3 and eFigure 6 in the Supplement). The average concentration of plasma phenytoin in the 90 phenytoin-tolerant controls was 11.8 μg/mL (95% CI, 11.0-12.6 μg/mL) (Table 3). The day of drug withdrawal in the 11 phenytoin-tolerant controls was 11.8 μg/mL (95% CI, 11.0-12.6 μg/mL).
tions were obtained before drug withdrawal because these patients were hospitalized and received phenytoin for seizure prophylaxis. Before drug withdrawal, plasma concentrations of phenytoin were significantly higher in patients with SJS-TEN (mean, 34 μg/mL; 95% CI, 1.8-66 μg/mL) compared with the phenytoin-tolerant controls (mean, 11 μg/mL; 95% CI, 9.1-13 μg/mL; \( P = .015 \)) (Table 3). After drug withdrawal for 1 to 5 days, concentrations of plasma phenytoin rapidly decreased in phenytoin-tolerant controls (mean, 2.5 μg/mL; 95% CI, 1.5-3.5 μg/mL) but remained significantly high in patients with SJS-TEN (mean, 12 μg/mL; 95% CI, 4.6-19 μg/mL; \( P = .0004 \)) and patients with DRESS (mean, 5.5 μg/mL; 95% CI, 2.8-8.3 μg/mL; \( P = .029 \)) (Table 3). Furthermore, significantly delayed clearance of plasma phenytoin was observed in patients with severe cutaneous adverse reactions with CYP2C9*3 (mean, 17 μg/mL; 95% CI, 5.9-27 μg/mL; \( P = .0002 \)) and in noncarriers (mean, 4.9 μg/mL; 95% CI, 3.1-6.7 μg/mL; \( P = .015 \)) (Table 3). The CYP2C9*2 carriers with severe cutaneous adverse reactions had significantly higher levels of plasma phenytoin than patients without the risk allele (\( P = .022 \)). However, the average daily dose showed no difference between patients with severe cutaneous adverse reactions carrying CYP2C9*3 (n = 12; mean, 300 mg/d; 95% CI, 300-300 mg/d) and noncarriers (n = 28; mean, 304 mg/d; 95% CI, 291-316 mg/d). These data suggest that rs1057910 (CYP2C9*3) contributes to phenytoin-related severe cutaneous adverse reactions.

Discussion

Phenytoin has a narrow therapeutic range (10-20 μg/mL) and nonlinear pharmacokinetics and is metabolized to inactive hydroxypyhenytoin, 5-(4′-hydroxyphenyl)-5-phenylhydantoin (p-HPHP), primarily (90%) by the cytochrome P450 (CYP) 2C9 enzyme. Formation of p-HPHP is thought to proceed via a reactive arene oxide intermediate, which has been proposed for the induction of phenytoin hypersensitivity. In this study, we report CYP2C variants, including CYP2C9*3, known to cause 93% to 95% reduction in phenytoin clearance, as important genetic factors for phenytoin-related severe cutaneous adverse reactions. We detected accumulated phenytoin in patients with severe cutaneous adverse reactions, particularly CYP2C9*3 carriers. Patients with SJS-TEN exhibited slower metabolism and a stronger association with the CYP2C SNPs than patients with DRESS. Delayed clearance was also noted in patients with severe cutaneous adverse reactions without CYP2C9*3, suggesting that nongenetic factors such as renal insufficiency, hepatic dysfunction, and concurrent use of substances that compete or inhibit the enzymes may also affect phenytoin metabolism and contribute to severe cutaneous adverse reactions. Such characteristics share the features of the drug-accumulation hypothesis of allopurinol-related severe cutaneous adverse reactions, in which the risk factors include high-dose regimen, renal failure, concomitant diuretic, and high concentration of oxypurinol. Further studies are needed to investigate how the CYP2C variants and the accumulated reactive metabolites affect cutaneous adverse reactions.

Among the 10 risk alleles, the missense rs1057910 is the only one with known function associated with reduced CYP2C9 enzyme activity and phenytoin-related neurological toxicity. The SNP rs1057910 forms CYP2C9*3 and part of CYP2C9*18. Another risk SNP, rs3758581, present on the CYP2C9*1B and CYP2C9*1C normal haplotypes, is a mis-sense mutation yet has no obvious effects on CYP2C19 activity or drug metabolism. The SNP rs3758581 may be a surrogate marker for rs1057910 because of the strong linkage disequilibrium between the 2 SNPs. In our 90 samples from patients with severe cutaneous adverse reactions, we did not detect CYP2C9*2 (rs1799853). The frequencies of CYP2C9*3 vary in ethnic groups (0.8%-10%). CYP2C9*3 was reported to be associated with phenytoin maculopapular exanthema (\( P = .007 \)) in Koreans. A GWAS using samples from 40 cases...
with maculopapular exanthema and 4 cases with severe cutaneous adverse reactions caused by phenytoin and 1296 controls from a British population failed to discover genome-wide significant variants; this may be explained by the limited sample size and maculopapular exanthema phenotype of most of the cases.

This study has several limitations. The sample sizes of severe cutaneous adverse reaction cases and phenytoin-tolerant controls were small, and we did not have samples from other population groups to replicate the genetic association. For the pharmacokinetic analysis, we had only a few available plasma samples, and most of the severe cutaneous adverse reaction samples were collected after drug withdrawal. Additionally, drug-tolerant participants were younger and more likely to be male than patients with severe cutaneous adverse reactions, which may account for some of the observed differences in drug metabolism.

This study highlights that genetic variants of metabolizing enzymes contribute to severe cutaneous adverse reactions, which is different from the previous HLA studies. Although the clinical manifestations and prognosis are quite different between SJS-TEN and DRESS, our data suggest some shared genetic factors. We propose that delayed clearance and accumulation of reactive metabolites caused by genetic variants of drug-metabolizing enzymes may be the primary factor, and that immunogenicity, such as the presence of risk HLA alleles and specific T-cell receptor clonotypes in susceptible individuals, may facilitate the development and guide the different types of cutaneous adverse reactions. Further investigation is required to determine how a complex interplay of impaired drug metabolism, accumulation of reactive drug compounds, HLA presentation of the drug/peptide antigens, T-cell receptor recognition, and historical immune memory triggers drug hypersensitivity.

Conclusions

This study identified CYP2C variants, including CYP2C9*3, known to reduce drug clearance, as important genetic factors associated with phenytoin-related severe cutaneous adverse reactions. These findings may have potential to improve the safety profile of phenytoin in clinical practice and offer the possibility of prospective testing for preventing phenytoin-related severe cutaneous adverse reactions. More research is required to replicate the genetic association in different populations and to determine the test characteristics and clinical utility.
Phenytoin-Related Severe Cutaneous Adverse Reactions

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Author Contributions: Drs Chung and Hung had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Dr Chung and Ms Chang contributed equally to this article as first authors. Study concept and design: Chung, Hung. Acquisition, analysis, or interpretation of data: All authors. Drafting of the manuscript: Chung, Hung. Critical revision of the manuscript for important intellectual content: All authors. Statistical analysis: Chung, W.-C., Chang, Lee, Shi, Y.-S. Chang. Hung. Obtained funding: Chung, Hung. Administrative, technical, or material support: All authors. Study supervision: Chung, Hung.

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Table 3. Comparison of Mean Concentrations of Plasma Phenytoin in Phenytoin-Tolerant Controls and Patients With Phenytoin-Related Severe Cutaneous Adverse Reactions

<table>
<thead>
<tr>
<th>Time Period</th>
<th>Phenytoin-Tolerant Controls</th>
<th>Cases</th>
<th>Severe Cutaneous Adverse Reactions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Continued Phenytoin Use</td>
<td>Discontinued Phenytoin Use</td>
<td>SJ-TEN (*)</td>
</tr>
<tr>
<td></td>
<td>(n = 90)</td>
<td>(n = 11)</td>
<td>(n = 14)</td>
</tr>
<tr>
<td>During continuous use of phenytoin</td>
<td>Phenytoin concentration, mean (95% CI) [range], μg/mL</td>
<td>11.8 (11.0-12.6) [5.6-20]</td>
<td>11.7 (9.9-12.6) [4.8-19]</td>
</tr>
<tr>
<td></td>
<td>P value</td>
<td>.02</td>
<td>.86</td>
</tr>
<tr>
<td>1 to 5 d after phenytoin withdrawal</td>
<td>No. of plasma samples a</td>
<td>32</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>Time since drug withdrawal, mean (95% CI), h</td>
<td>62 (50-73)</td>
<td>65 (48-82)</td>
</tr>
<tr>
<td></td>
<td>Phenytoin concentration, mean (95% CI) [range], μg/mL</td>
<td>2.5 (1.5-3.5) [0-12]</td>
<td>12 (4.6-19) [0.8-46]</td>
</tr>
<tr>
<td></td>
<td>P value</td>
<td>4.0×10⁻³ b</td>
<td>.029</td>
</tr>
<tr>
<td>&gt;5 d after phenytoin withdrawal</td>
<td>No. of plasma samples a</td>
<td>4</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>Time since drug withdrawal, mean (95% CI), h</td>
<td>168 (137-199)</td>
<td>278 (229-328)</td>
</tr>
<tr>
<td></td>
<td>Phenytoin concentration, mean (95% CI) [range], μg/mL</td>
<td>0.3 (-0.7-1.3) [0-1.2]</td>
<td>3.3 (0.5-6.2) [0-21]</td>
</tr>
<tr>
<td></td>
<td>P value</td>
<td>.33</td>
<td>.44</td>
</tr>
</tbody>
</table>

Abbreviations: DRESS, drug reaction with eosinophilia and systemic symptoms; SJ-TEN, Stevens-Johnson syndrome; TEN, toxic epidermal necrolysis.

a The plasma samples for the pharmacokinetic analysis were based on sample availability; more than 1 sample could be obtained from a particular participant at different time points.

b P values were calculated by nonparametric tests for the comparison between the difference of plasma phenytoin concentrations in the cases and 11 phenytoin-tolerant controls.

A nonparametric test was used to examine the differences in the time interval after drug withdrawal among the samples obtained from the subgroups. No significant difference in time intervals was found between the subgroups of cases and 11 phenytoin-tolerant controls.
pending for risk assessment for phenytoin-induced adverse drug reactions. No other disclosures were reported.

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