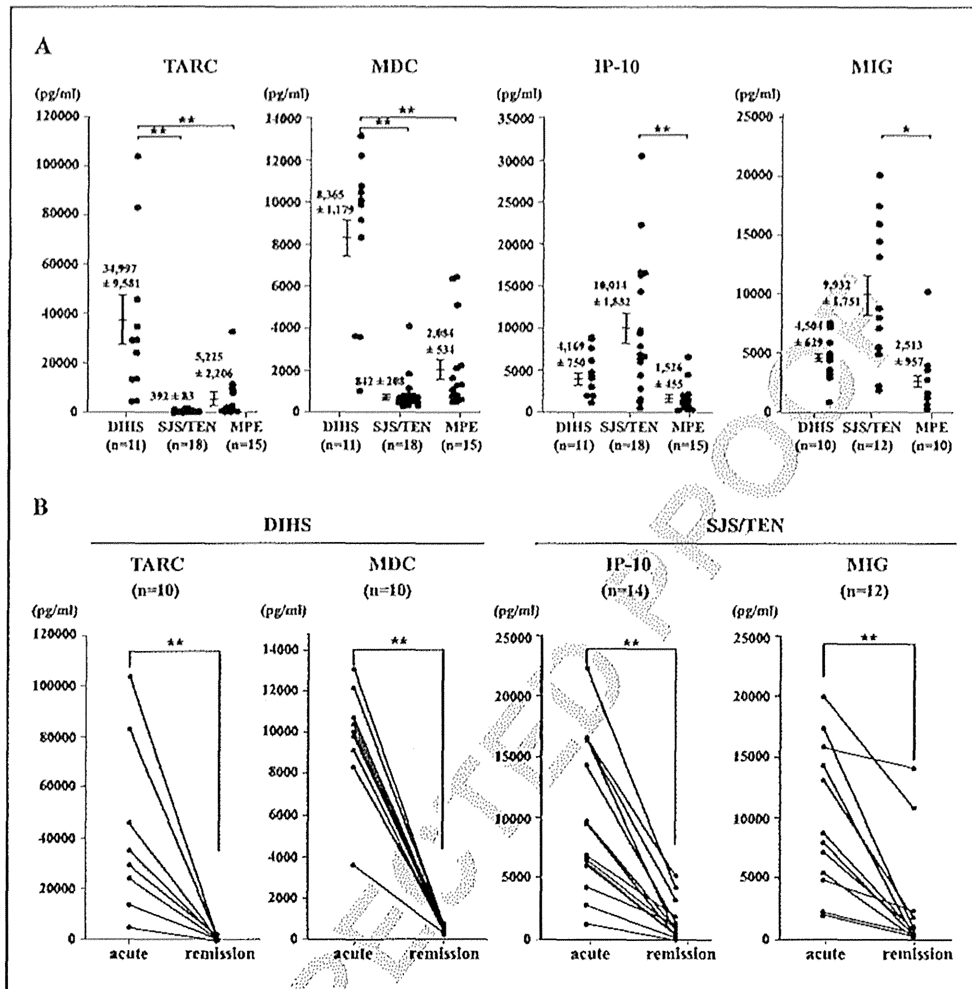

Figure 1. A) Distinct expression of chemokines in SCAR in the acute stage. Th2 chemokines (TARC and MDC) were highly elevated in DIHS/DRESS, while Th1 chemokines (IP-10 and MIG) predominated in SJS/TEN. The average TARC and MDC levels in patients with DIHS/DRESS were 34,997 ± 9,581 pg/mL (average ± SEM) and 8,365 ± 1,179 pg/mL, respectively. The average levels of IP-10 and MIG in patients with SJS/TEN were 10,014 ± 1,882 pg/mL and 9,932 ± 1,751 pg/mL, respectively. Blood samples were obtained on days 0-31 (average day 8.8) after the onset of DIHS/DRESS, days 0-25 (average day 5.6) for SJS/TEN, and days 1-24 (average day 6.5) for MPE. * $p<0.05$, ** $p<0.01$, Kruskal-Wallis test. B) Serum chemokine levels in the acute and remission stages in SCAR. The levels of chemokines that were upregulated during the acute stage (TARC and MDC in patients with DIHS, and IP-10 and MIG in patients with SJS/TEN) declined upon remission. ** $p<0.01$, Student's *t*-test.

underlying DIHS/DRESS and SJS/TEN are distinct. We further suggest that a prompt differentiation of SCAR may be achieved using TARC/MDC and IP-10/MIG chemokine sets. ■

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Letters to the Editor

Drug-induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms with histologic features mimicking cutaneous pseudolymphoma

Dear Editor,

Drug-induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms (DIHS/DRESS) may be included in the broad classification of drug-induced pseudolymphoma. However, these conditions seem to be two distinct entities, with different clinical features and outcomes.[1] We report a case of DIHS/DRESS showing histologic features of cutaneous pseudolymphoma.

A 31-year-old man presented with a 10-day history of high fever and generalized rash. He suffered from bipolar depression and had been treated with sodium valproate. Twenty-one days after adding carbamazepine, high fever developed, followed by

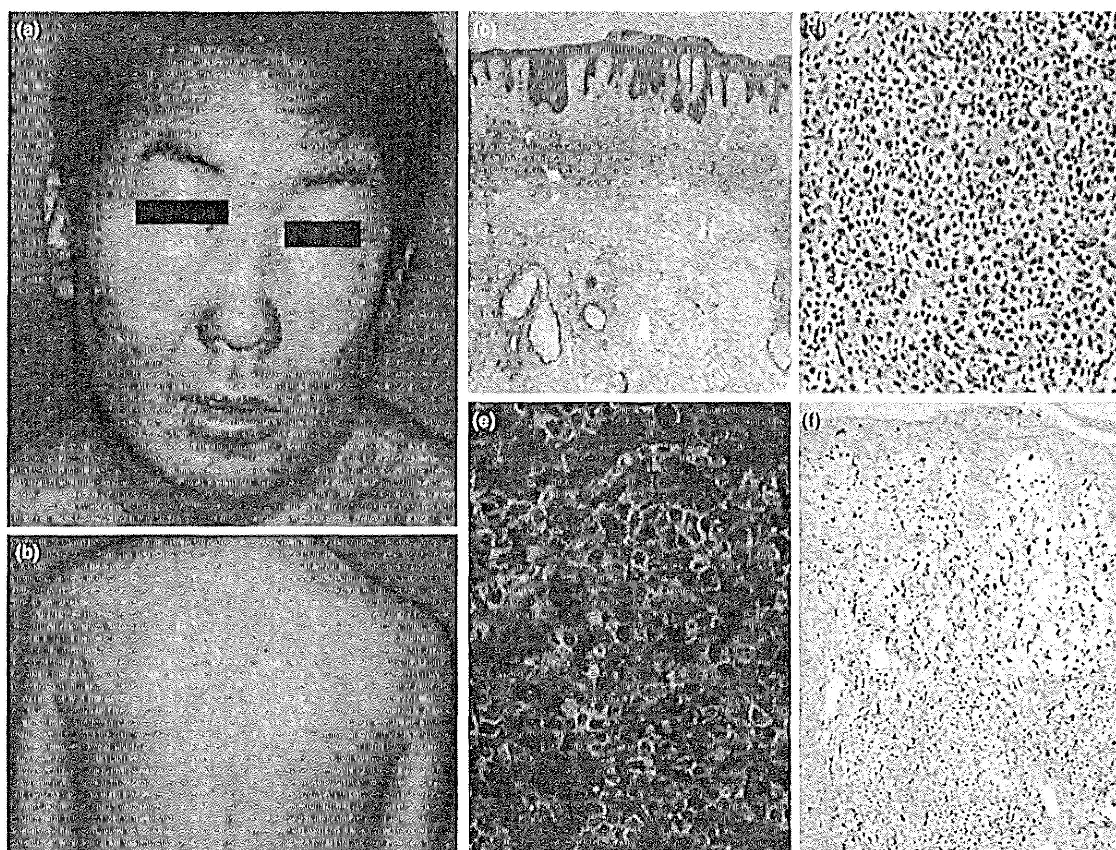

Figure 1. (a, b) Patient clinical features. Erythroderma with edematous swelling of the face. (c, d) Histology of the neck skin. Well-demarcated patches of infiltrates around the dermal vessels and folliculosebaceous units (c: hematoxylin-eosin, 40×). Dense infiltrates consisted of lymphoid cells predominantly with occasional atypical nuclei and minimal nuclear debris (d: hematoxylin-eosin, 400×). (e) Double immunofluorescence labeling of CD3 (green) and FoxP3 (red) revealed 10 double-positive cells in infiltrates per high-power field (1000×). (f) Large numbers of $CD16^{+}$ monocytes were observed in edematous dermal papillae and the peripheries of the dense infiltrates (100×).

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extensive erythematous plaques, lymphadenopathy and liver dysfunction. Examination revealed erythroderma on the whole body (Fig. 1a,b). Cervical, axillary, and inguinal lymph nodes were swollen. Sodium valproate and carbamazepine were discontinued, and the patient was admitted for suspected DIHS/DRESS. Laboratory findings on admission were as follows: white blood cell count 29 400/μL (eosinophils 7%, atypical lymphocytes 34%); AST 119 IU/mL; ALT 318 IU/mL; LDH 1105 IU/mL; γ-GTP 806 IU/mL; and soluble IL-2 receptor 2130 U/mL. Histology of the neck skin revealed edematous dermal papillae and well-demarcated patches of infiltrates around the dermal vessels and folliculosebaceous units. Dense infiltrates consisted predominantly of lymphoid cells with occasional atypical nuclei (Fig. 1c,d). By immunohistochemistry, most infiltrates were $CD3^+$ T-cells with higher expression of $CD8^+$ than $CD4^+$. Double immunofluorescence labeling of infiltrates revealed 7–10 $CD3^+FoxP3^+$ cells per high-power field (Fig. 1e). Cells labeled for B cell markers such as CD20 and CD79a were few. Large numbers of $CD16^+$ monocytes were distributed in edematous dermal papillae and the peripheries of the dense infiltrates (Fig. 1f), whereas $CD14^+$ cells were few, as reported in a DIHS/DRESS case series.[2] Prednisone (0.7 mg/kg/day, drip infusion) was started and slowly tapered. Serum HHV-6 DNA was detected 20 days after the onset of disease. Anti-HHV-6 IgG titers increased significantly (from 40× to 2560×) in paired sera. A drug-induced lymphocyte stimulation test performed at day 112 of the disease course was positive for carbamazepine (SI index 19.51) and sodium valproate (SI index 3.53).

Callot *et al.*[1] endeavored to separate the two conditions retrospectively, based on 24 cases and an additional 95 published cases. The pseudolymphoma group demonstrated subacute papulonodular or infiltrated plaques devoid of visceral involvement.[1] Histology revealed dense lymphocytic infiltrates and occasional Pautrier's microabscess mimicking lymphoma.[3,4] In contrast, the DIHS/DRESS group had acute widespread rash with high fever, lymphadenopathy, and multivisceral involvement.[1] Systemic involvement, such as extensive lymphadenopathy and peripheral blood leukocytosis with atypical lymphocytosis were suggestive of pseudolymphoma, whereas histology of the skin was usually not specific. In the present case, the clinical manifestation was DIHS/DRESS, while the histology mimicked pseudolymphoma. This case suggested some features of pseudolymphoma in DIHS/DRESS, although the two entities are distinct. Dramatic expansion of functional regulatory T-cells (Tregs) in peripheral blood and abundant Tregs in the dermis of DIHS/DRESS patients have been reported.[5] A number of $CD3^+FoxP^+$ cells, presumably Tregs, were found in the dense infiltrates in our case. Although the ratio to $CD3^+$ cells was not higher than for usual DIHS/DRESS, Tregs might serve to prevent the further activation and expansion of effector T cells.

CONFLICT OF INTEREST: None.

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Immediate-onset anaphylaxis of *Bacillus subtilis*-fermented soybeans (*natto*)

Dear Editor,

I read the Letter to the Editor entitled "Involvement of poly (γ-glutamic acid) as an allergen in late-onset anaphylaxis due to fermented soybeans (*natto*)" by Inomata *et al.*[1] with interest. *Natto* is a traditional Japanese preserved food made by fermenting soy beans with *Bacillus subtilis natto*, a type of hay bacillus. In recent years, there have been increasing opportunities to consume *natto* throughout the world, due to the boom in health foods and Japanese foods. Unlike ordinary food allergies, *natto* anaphylaxis is distinguished by symptoms that appear with a delay of 5–12 h after ingesting *natto*.[1–4] To our knowledge, we report the first case of immediate-onset ana-

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lidomide may be considered as a therapeutic option in resistant cases of OFG as long as regular neurological assessment is carried out.

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Elevated serum thymus and activation-regulated chemokine (TARC/CCL17) relates to reactivation of human herpesvirus 6 in drug reaction with eosinophilia and systemic symptoms (DRESS)/drug-induced hypersensitivity syndrome (DIHS)

DOI: 10.1111/bjd.12948

Dear Editor, Drug reaction with eosinophilia and systemic symptoms (DRESS), also known as drug-induced hypersensitivity syndrome (DIHS), is a severe adverse drug-induced reaction. This syndrome is characterized by cutaneous eruptions, fever, haematological abnormalities (eosinophilia and/or atypical lymphocytosis) and severe visceral dysfunction.[1,2] The reactivation of human herpesvirus (HHV)-6, as evidenced by increases in HHV-6 IgG antibody titres and DNA levels, has been reported in patients with DRESS/DIHS.[2–4] It is often challenging to diagnose DRESS/DIHS due to its diverse symptoms; therefore, a scoring system (RegiSCAR system) has recently been developed in an attempt to define DRESS more appropriately.[5]

Thymus and activation-regulated chemokine (TARC/CCL17) is one of the C–C chemokines that works as a ligand for C–C chemokine receptor 4, and plays important roles in the T helper 2-type immune response.[6,7] We recently reported markedly higher serum TARC levels in patients with

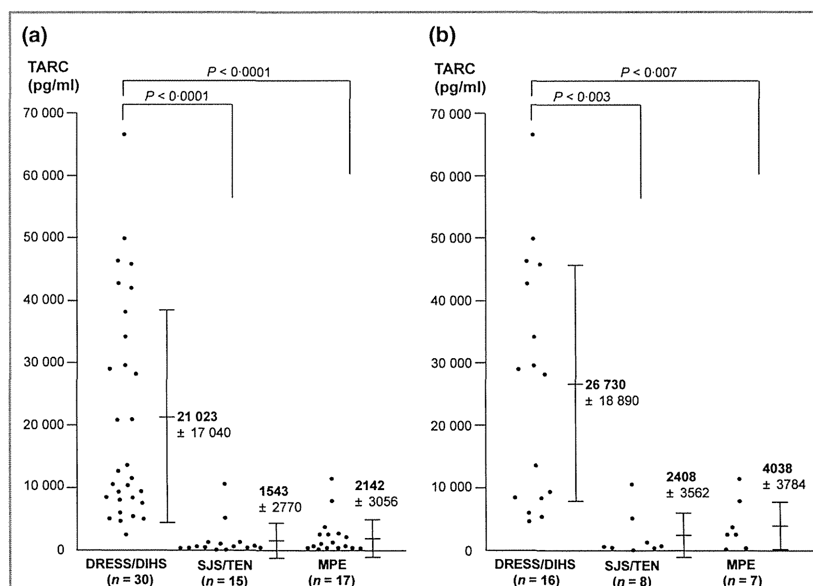

Fig 1. (a) Serum thymus and activation-regulated chemokine (TARC) levels in the acute stage of drug eruption within 15 days of onset. TARC levels in patients with drug reaction with eosinophilia and systemic symptoms (DRESS)/drug-induced hypersensitivity syndrome (DIHS) were significantly higher than those in patients with Stevens–Johnson syndrome/toxic epidermal necrolysis (SJS/TEN) or maculopapular erythema (MPE). (b) Serum TARC levels in patients with drug eruption between days 5 and 10 after onset. TARC levels in patients with DRESS/DIHS were significantly higher than those in patients with SJS/TEN or MPE.

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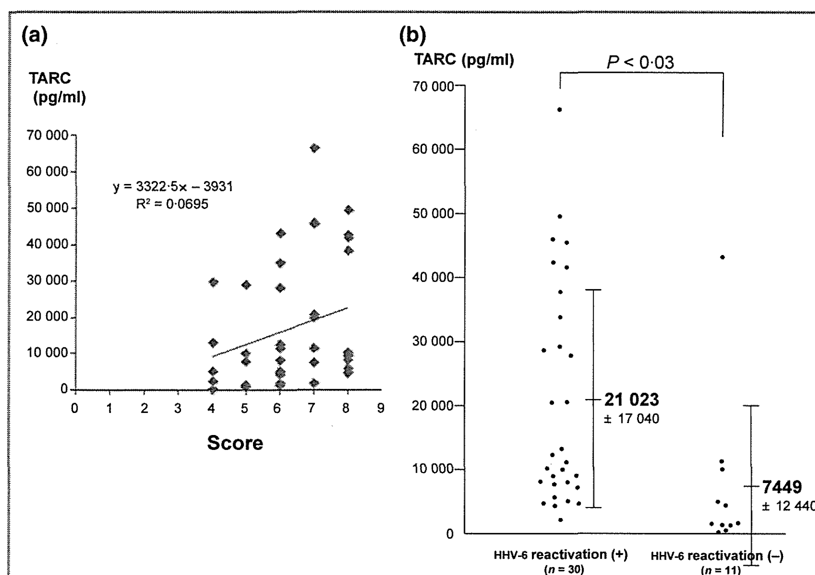

Fig 2. (a) Correlation between the RegiSCAR group diagnostic score for drug reaction with eosinophilia and systemic symptoms (DRESS) and serum thymus and activation-regulated chemokine (TARC) levels in patients with DRESS. (b) Serum TARC levels in 30 patients with DRESS with human herpesvirus (HHV)-6 reactivation were significantly higher than those in 11 patients with DRESS without HHV-6 reactivation ($P < 0{\cdot}03$).

DRESS/DIHS than in patients with other forms of drug eruptions including Stevens–Johnson syndrome/toxic epidermal necrolysis (SJS/TEN) and maculopapular erythema (MPE).[8] Serum TARC levels in the acute stage of DRESS/DIHS have been correlated with disease activity; therefore, we proposed that DRESS/DIHS may be diagnosed early by measuring serum TARC levels. In the present study we confirmed our previous findings using a larger sample of patients with DRESS/DIHS. We also showed that the increase in serum TARC levels may be associated with HHV-6 reactivation.

We evaluated 30 patients diagnosed with DRESS/DIHS associated with HHV-6 reactivation (22 male and eight female; median age 52.4 years, range 12–86 years). Fifteen patients with SJS/TEN (eight male and seven female; median age 57·7 years, range 33–78 years) and 17 patients with MPE (10 male and seven female; median age 61.2 years, range 32–77 years) were also enrolled in this study. We evaluated serum TARC levels in patients with active skin eruptions in the acute stage within 15 days of onset (average day 10.0). Serum samples of SJS/TEN and MPE were obtained on days 0–10 (average day 4.7) and days 1–11 (average day 4.8), respectively. Serum TARC levels were measured using enzyme-linked immunosorbent assays (R&D Systems, Minneapolis, MN, U.S.A.). The results obtained showed that the mean ± SD serum TARC levels in patients with DRESS/DIHS (21 023 ± 17 040 pg mL^{-1}) were significantly higher than those in patients with SJS/TEN (1543 ± 2770 pg mL^{-1}) or MPE (2142 ± 3056 pg mL^{-1}), which was consistent with our previous findings (Fig. 1).[8] To exclude the possibility that the major differences in TARC levels between the three groups were due to the difference in sampling time, we next analysed the samples that were obtained from day 5 to day 10 after onset. Sixteen cases of DRESS, eight of SJS/TEN and seven of MPE fulfilled this criterion. As shown in Figure 1b, mean serum TARC levels in patients with DRESS/DIHS (26 730 ± 18 890 pg mL^{-1}) were significantly higher than those in patients with SJS/TEN (2408 ± 3562 pg mL^{-1}) or MPE (4038 ± 3784 pg mL^{-1}) even if we included only the samples obtained between day 5 and day 10 after onset.

We then investigated whether serum TARC levels correlated with the RegiSCAR group diagnostic score for DRESS[5] and HHV-6 reactivation. This study included 41 patients suspected of having DRESS/DIHS due to their clinical symptoms, regardless of HHV-6 reactivation (28 male and 13 female; median age 51·0 years, range 12–86 years). Thirty patients, the same patient population as that in Figure 1a, had HHV-6 reactivation, while the other 11 cases showed no evidence of HHV-6 reactivation. Forty-one patients were graded according to the RegiSCAR scoring system as 'probable' ($n = 10$) or 'definite' ($n = 31$). Serum samples were obtained during the acute stage, within 15 days of onset. A weak correlation was found between serum TARC levels and DRESS scores in 41 patients ($r = 0{\cdot}26$; Fig. 2a). Serum TARC levels in patients with HHV-6 reactivation (21 023 ± 17 040 pg mL^{-1}) were significantly higher than those in patients without HHV-6 reactivation (7449 ± 12 440 pg mL^{-1}) (Fig. 2b).

The present study confirms that serum TARC levels can be a useful indicator to differentiate DRESS/DIHS with HHV-6 reactivation from other drug eruptions, as we have reported previously.[8] We also demonstrated that serum TARC levels in patients with HHV-6 reactivation were higher than those in patients without HHV-6 reactivation. This finding led us to suggest the pathogenic link between serum TARC levels and HHV-6 reactivation. Although the precise mechanism involved is largely unknown, one possible explanation is that immunosuppression can trigger HHV-6 reactivation through the process of regulatory T-cell activation induced by elevated TARC. Another possibility is that elevated TARC levels directly activate HHV-6 through the chemokine receptor homologues of

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HHV-6. Further studies are warranted to address this mechanism.

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Conflicts of interest: none declared.

A novel mutation for disseminated superficial actinic porokeratosis in the *MVK* gene

DOI: 10.1111/bjd.12947

Dear Editor, Disseminated superficial actinic porokeratosis (DSAP) is the most common clinical type of porokeratosis, characterized by many uniformly small, minimal, annular, anhidrotic and keratotic lesions developing on patients' sun-exposed areas (e.g. face, neck, arms and legs) (Fig. 1a,b).[1] A four-generation family with DSAP (Fig. 2a), consisting of 27 individuals (six affected), was identified in Hunan province in China. The lesions had developed between the ages of 20 and 28 years. Diagnosis of DSAP in each case was made by an experienced dermatologist based on clinical examination of the patient, and was confirmed by histological findings of involved skin. Skin biopsy samples of one patient (III-3) were obtained for histological examination (Fig. 1c,d). Biopsy of individual III-3 revealed a typical cornoid lamella in the epidermis. The granular layer was absent or decreased under the parakeratotic column. Peripheral blood samples of 14 individuals for DNA isolation were collected following informed consent and institutional ethical committee approval. Laboratory examinations (blood, urine, routine liver and renal function) were normal. Genomic DNA was isolated from the peripheral blood of the family members by standard methods. All 11 translated exons plus the flanking splice sites of the *MVK* gene (located at 12q24, containing 10 coding exons and one noncoding exon spanning over 21 kb) were amplified from the genomic DNA of the proband (III-3) using Premix LA Taq (TaKaRa Biotechnology Co., Dalian, China).[2] The primer sequences are presented in Table 1. The polymerase chain reaction (PCR) conditions are available on request. The amplified PCR products were subjected to direct sequencing on an ABI 3730xl Automated Sequencer, using the ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA, U.S.A.). After identification of the mutation in the proband, genotyping of all included individuals was carried out by PCR amplification and direct sequencing of exon 10 of the *MVK* gene.

DNA analysis of the proband showed a heterozygous substitution of guanine for thymine in exon 10 of *MVK*, resulting in the replacement of glycine with the more hydrophobic valine in the MVK protein (c.926G>T, p.Gly309Val) (Fig. 2b). The missense mutation was detected in all affected individuals and one asymptomatic 6-year-old girl (IV-2) in the heterozygous state, but was not detected in unaffected family members, which provided evidence for cosegregation of the mutation with the DSAP phenotype in this family. The asymptomatic carrier in this study is only 6 years old, which shows the age-related penetrance of DSAP. Furthermore, the missense mutation was absent in 100 ethnically matched controls, suggesting that the mutation was not polymorphous.

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Letter to the Editor

TNF-α as a useful predictor of human herpesvirus-6 reactivation and indicator of the disease process in drug-induced hypersensitivity syndrome (DIHS)/drug reaction with eosinophilia and systemic symptoms (DRESS)

Dear Editor,

Drug-induced hypersensitivity syndrome (DIHS), which is also referred to as drug reaction with eosinophilia and systemic symptoms (DRESS), is a multi-organ systemic reaction characterized by rashes, fever, leukocytosis with eosinophilia and atypical lymphocytes, liver dysfunction, and reactivation of human herpesvirus-6 (HHV-6) [1–4]. The mortality rate of DIHS/DRESS has recently been demonstrated to be 2–14% [3,4]. However, the pathogenesis of this serious syndrome has not been fully elucidated.

Whether reactivation of members of the Betaherpesvirinae subfamily, including HHV-6, occurs subsequent to drug hypersensitivity reactions is one of the major clinical focuses in diagnosis of DIHS/DRESS and selecting the most appropriate treatment for better outcomes in patients [1]. However, a useful, predictive marker of HHV-6 reactivation has not been widely accepted. Moreover, useful biomarkers that reflect the disease process of DIHS/DRESS have not been reported. Therefore, we conducted comparative assessments and detailed examinations of patients with DIHS/DRESS and measured their serum protein levels. We compared their serum levels with those of patients with other types of drug eruptions, such as erythema multiforme (EM) due to drugs/medications and Stevens-Johnson syndrome (SJS)/toxic epidermal necrolysis (TEN).

This study was approved by the Ethics Committee of Showa University School of Medicine. Diagnosis of DIHS/DRESS was determined according to the criteria established by the Japanese consensus group [1]. Diagnosis of SJS, overlap SJS/TEN, and TEN were performed according to criteria reported by Bastuji-Garin et al. [5]. Regarding selection of subjects for the study, 20, 4, and 7 patients who satisfied the full criteria for DIHS/DRESS, SJS/TEN (with an overlap of SJS/TEN patients; $n = 2$, and TEN patients; $n = 2$), and EM caused by drugs/medications [1,6] respectively, and for whom sufficient laboratory data were available. The dermatological manifestations of DIHS/DRESS are as follows: maculopapular rash-type, EM-type, and erythroderma [1,3]. Among the 20 DIHS/DRESS patients, there were 7 cases of maculopapular rash-type, 5 of EM-type, and 8 of erythroderma (Table 1).

In DIHS/DRESS cases, HHV-6 infection was evaluated by serum sample serological tests on admission and at various times thereafter. Titers of IgG and IgM to HHV-6 were determined using an indirect immunofluorescence antibody assay in all DIHS/DRESS patients, and serum HHV-6 DNA was measured in 18 of 20 patients using real-time polymerase chain reaction (PCR) [7]. We determined the serum levels of interleukin (IL)-6, tumor necrosis factor (TNF)-α, and IL-13 on admission and after recovery and compared them with those in patients with EM and SJS/TEN.

Anti-HHV-6 IgG titers were significantly increased in 13 of the 20 DIHS/DRESS patients. The presence of HHV-6 DNA in the serum of 18 patients was determined, and 10 patients tested positive for HHV-6 DNA (mean, 25.1 ± 7.5 days after onset in nine patients). In one patient in whom HHV-6 DNA was detected (Pt. 3, Table 1), anti-HHV-6 IgG titers were not increased significantly.

HHV-6 belongs to the Betaherpesvirinae subfamily, which contains two additional human herpesviruses: cytomegalovirus (CMV) and HHV-7 [1]. Previously, increased levels of proinflammatory cytokines such as TNF-α and IL-6 have been demonstrated with HHV-6 and CMV infection [8,9]. However, the exact mechanisms of reactivation of these viruses have not been fully elucidated. We determined the serum levels of TNF-α, IL-6, IL-13, C-reactive protein (CRP), and lactate dehydrogenase (LDH) in 14 patients with DIHS/DRESS whose serum had been stored for protein analysis on admission (Table 1), and compared these results between the HHV-6 reactivation and non-reactivation groups. The serum levels of TNF-α, CRP, and LDH before treatment were significantly higher in the HHV-6 reactivation group than in the HHV-6 non-reactivation group (TNF-α, $P = 0.0220$; CRP, $P = 0.0264$; LDH, $P = 0.0341$) (Fig. 1A–C). In our study, a TNF-α level of 12 pg/mL, a CRP level of 7 mg/dl, and a LDH level of 600 U/L were sufficient for detection of HHV-6 reactivation. Eight of fourteen patients satisfied the threshold of TNF-α. Levels of other proteins upon admission were not significantly correlated with either group. IL-13 was undetectable in the sera of all subjects.

Regarding conditions similar to DIHS/DRESS, Kamijima et al. recently reported the investigation of 28 patients with trichloroethylene hypersensitivity syndrome, including the reaction point of onset after exposure to trichloroethylene/drugs, clinical manifestations, blood examination, and period of virus reactivation [10]. They found that an elevated TNF-α level on admission was significantly correlated with an increase in HHV-6 DNA during the clinical course. This result supports our observation that an increasing level of TNF-α prior to the commencement of treatment may be an excellent biomarker for the early recognition of HHV-6 reactivation in patients with DIHS/DRESS.

Moreover, the TNF-α, CRP, and LDH levels decreased significantly in parallel with the response to treatment in only the DIHS/DRESS group (TNF-α, $P = 0.0418$; CRP, $P = 0.0001$; LDH, $P = 0.0026$) (Fig. 1D–F). To date, there have been no widely accepted biomarkers of the DIHS/DRESS disease process. Yoshikawa et al. reported elevated TNF-α levels in four of six DIHS/DRESS patients at onset [9]. These results indicate that the serum levels of these proteins reflect the DIHS/DRESS disease process; however, further investigation using a larger number of DIHS/DRESS samples is required.

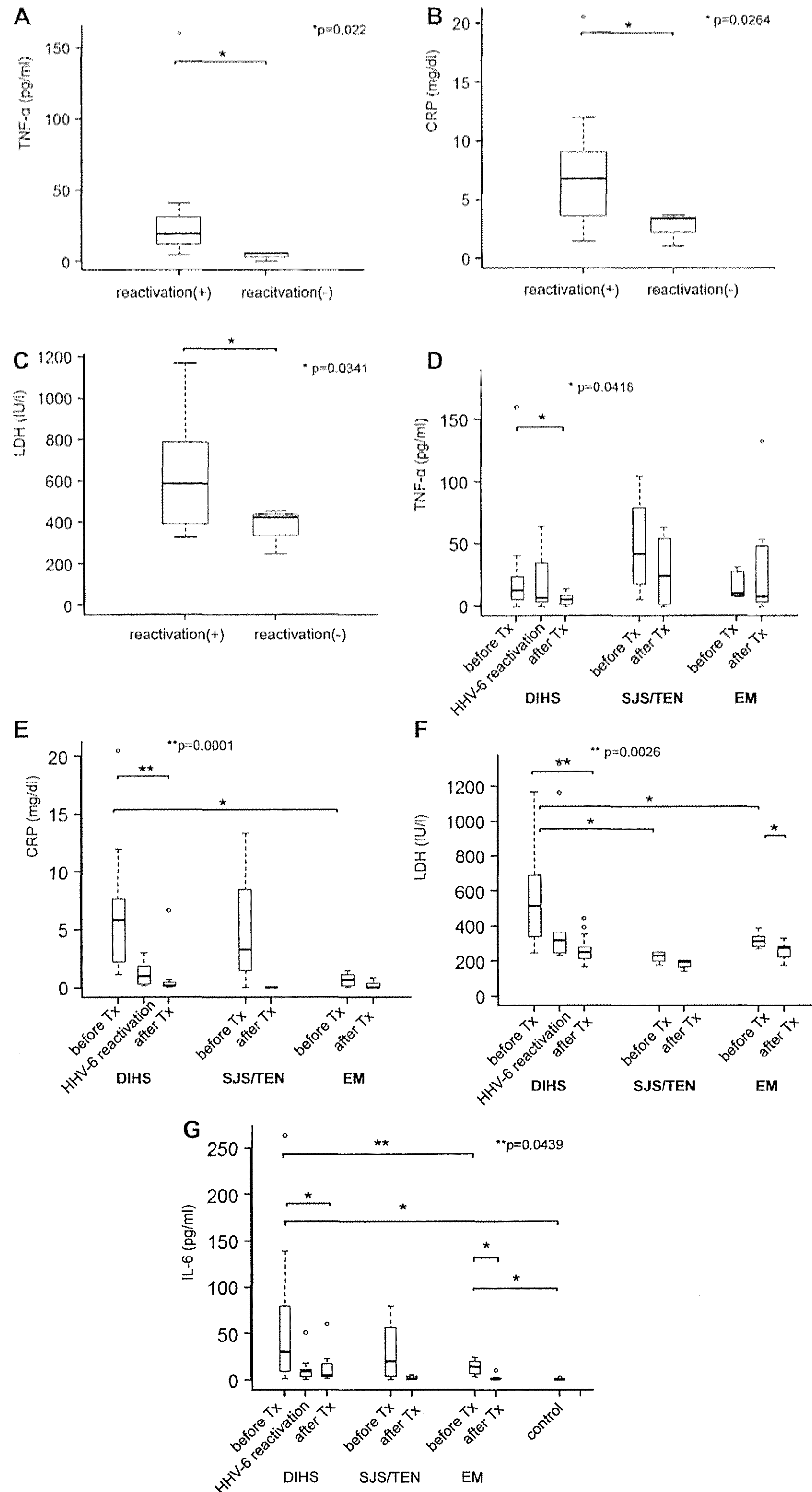

Fig. 1. Protein levels in the HHV-6 reactivation group and non-HHV-6 reactivation group in DIHS/DRESS ($n = 14$). In the DIHS/DRESS group, serum levels of TNF-α ($^*P < 0.05$) (Fig. 1A), CRP ($^*P < 0.05$) (Fig. 1B), and LDH ($^*P < 0.05$) (Fig. 1C) before treatment were significantly higher in the HHV-6 reactivation group than in the non-HHV-6 reactivation group. TNF-α, LDH, and CRP levels decreased significantly in parallel with the response to treatment in only the DIHS/DRESS group ($^*P < 0.05$) (Fig. 1D–F). The serum IL-6 levels on admission were significantly higher in the DIHS/DRESS group than in the EM group ($^*P < 0.05$) (Fig. 1G). The *t*-test and Mann–Whitney *U*-test were applied to evaluate differences in serum levels between the two groups. Regression analysis was also performed to elucidate trends in treatment responses. Correlations between two serum levels were examined using Pearson's correlation test. A *P*-value of <0.05 was considered to indicate statistical significance for all tests.

Table 1
Patient data (demographics, causative drugs, onset of symptoms, type of cutaneous eruption, systemic involvement, and HHV-6 reactivation).

Pt.	Age	Sex	Causative drug	Onset (days)	Type of eruption	Leukocytosis (/μl)	Eosinophilia (/mm³)	Aty lym (%)	ALT (IU/L)	Cr (mg/dl)	HHV-6 reactivation	TNF-α (pg/ml)	CRP (mg/dl)	LDH (IU/l)
1	54	M	Phenytoin	41	Maculopapular	Yes (48,000)	Yes (3220)	Yes (3)	Yes (721)	Yes (6.63)	No	n.d.	n.d.	n.d.
2	35	F	Salazosulfapyridine	23	Maculopapular	Yes (13,700)	Yes (1781)	Yes (3)	Yes (1084)	Yes (1.13)	Yes	21.6	10.39	1005
3	65	M	Carbamazepine	58	EM-type	Yes (10,300)	No	No	Yes (81)	No	Yes	12.4	20.54	445
4	46	M	Carbamazepine	13	EM-type	Yes (10,600)	No (1038)	Yes (1)	Yes (87)	No	Yes	n.d.	n.d.	n.d.
5	32	M	Carbamazepine	21	Erythroderma	Yes (10,200)	No (1200)	Yes (14)	Yes (450)	No	No	n.d.	n.d.	n.d.
6	42	M	Carbamazepine	33	Maculopapular	Yes (16,900)	No (1183)	Yes (25)	Yes (131)	No	No	0	3.7	248
7	34	M	Phenobarbital	33	Maculopapular	Yes (10,700)	No	Yes (33.5)	Yes (890)	No	No	5.7502	3.4	425
8	27	M	Phenobarbital	14	Maculopapular	Yes (18,900)	Yes (2457)	Yes (17)	Yes (1156)	No	Yes	40.96	2.26	692
9	51	M	Allopurinol	12	Erythroderma	Yes (10,100)	No (441)	Yes (1)	Yes (118)	No	Yes	24.125	6.6	338
10	58	M	Carbamazepine	33	Erythroderma	Yes (16,800)	Yes (4872)	Yes (14)	Yes (242)	Yes (1.7)	Yes	13.075	6.8	883
11	49	M	Allopurinol	32	Erythroderma	Yes (17,100)	Yes (7182)	Yes (24)	Yes (302)	Yes (1.4)	Yes	38.819	7.7	588
12	86	M	Allopurinol	28	EM-type	Yes (17,500)	Yes (7500)	Yes (9)	Yes (65)	Yes (1.6)	Yes	19.678	5.1	584
13	54	F	Carbamazepine	27	Erythroderma	Yes (22,300)	Yes (1806)	Yes (24)	Yes (422)	Yes (1.5)	Yes	n.d.	n.d.	n.d.
14	74	M	Carbamazepine	35	Erythroderma	Yes (40,000)	Yes (4400)	Yes (3)	Yes (56)	No	Yes	10.133	6.9	344
15	69	M	Carbamazepine	30	EM-type	Yes (13,400)	Yes (1500)	Yes (1)	Yes (86)	No	Yes	4.494	12	326
16	47	M	Carbamazepine	78	Erythroderma	Yes (23,500)	Yes (8225)	Yes (6)	Yes (112)	No	Yes	n.d.	n.d.	n.d.
17	35	M	Carbamazepine	12	EM-type	Yes (15,000)	Yes (1500)	Yes (7)	Yes (213)	No	No	5.8931	1.1	453
18	30	M	Trichloroethylene	21	Erythroderma	Yes (21,700)	Yes (5425)	Yes (13)	Yes (391)	Yes (2.0)	Yes	159.864	1.8	659
29	45	F	Carbamazepine	31	Maculopapular	Yes (12,100)	No (630)	No	Yes (139)	No	No	n.d.	n.d.	n.d.
20	55	F	Mexiletine	50	Maculopapular	Yes (15,100)	Yes (9966)	Yes (23)	Yes (75)	No	Yes	11.748	1.5	1168

All patients with a diagnosis of DIHS/DRESS were treated at Showa University Hospital, Department of Dermatology, between August 2001 and March 2013. The study population comprised 16 males and 4 females ranging in age at the time of the initial examination from 27 to 86 years, with a mean age of 49.3 ± 15.7 years in the DIHS/DRESS group. The mean duration from the initial exposure to the suspected medication until the onset of DIHS/DRESS was 31.2 ± 16.3 days. White blood cell counts exceeding 11,000/μL (normal range 3500–9000/μL) at the initial examination were found in 15 patients (75%). During the clinical course, eosinophilia (≥1500/mm³; normal range 70–440/μL) was noted in 13 patients (65%). Atypical lymphocytes were found in 18 of 20 patients (90%), and 12 of 20 patients (60%) had >5% atypical lymphocytes. All patients had hepatic abnormalities (alanine aminotransferase (ALT) above the normal range of 5–25 IU/L), and 14 patients (70%) had a serum ALT >100 IU/L. Seven patients (35%) had renal dysfunction, one of whom was on continuous dialysis. HHV-6 DNA of Pt. 16 was positive during the entire course of their illness because of chromosomal integration of HHV-6 DNA.
CRP; C-reactive protein (normal range <0.2 mg/dl), LDH; lactate dehydrogenase (normal range 105–220 U/L).
Onset (day), onset of symptoms (day); Aty Lym (%), Atypical lymphocytosis (%); ALT (IU/L), hepatitis (maximum of ALT (IU/L)); Cr (mg/dl), renal impairment (Cr (mg/dl)); eosinophilia (/mm³), eosinophilia (≥1500/mm³); n.d., not done.

Finally, the serum IL-6 levels on admission were significantly higher in the DIHS/DRESS group than in the EM group ($P = 0.0439$) (Fig. 1G). Some DIHS/DRESS patients manifest targetoid erythematous lesions; such patients may be clinically similar to those with EM due to drugs/medications, especially in the early stage of the disease course. In fact, 5 of 20 patients showed targetoid erythematous skin manifestations. Based on our investigation, IL-6 could be a good marker for the early recognition of DIHS compared with EM.

In conclusion, we herein present a large case series involving a single-facility survey of DIHS/DRESS in Japan. This study suggests that elevated TNF-α during the early onset stage is a good marker for the early recognition of HHV-6 reactivation. The TNF-α level also reflects therapeutic responses and could be a useful marker of the DIHS/DRESS disease process. Early and careful recognition of these factors make it possible to choose an appropriate treatment and improve patient outcomes.

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SHORT COMMUNICATION

Development of Necrotising Fasciitis in a Patient Treated for Rheumatoid Arthritis with Tocilizumab

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Necrotising fasciitis (NF) is a potentially lethal infection affecting subcutaneous tissue and superficial fascia that may progress to multiple organ failure, and the prognosis is often worse when the host is immunocompromised. The prognosis is influenced by early diagnosis and early surgical debridement (1). Biologic therapies have been used in treating intractable inflammatory diseases, including collagen disorders. When using such a treatment strategy, it is critical to be aware that the treated subject may be highly susceptible to infection. Tocilizumab is a humanised monoclonal antibody directed against the interleukin-6 (IL-6) receptor and is recognised as an excellent biologic treatment in inflammatory rheumatic conditions (2). However, we wish to highlight the fact that its use may be associated with serious adverse reactions such as NF. It is known that tocilizumab may completely suppress induced C-reactive protein (CRP) via neutralisation of IL-6 effects (3). In fact, there have been a few reports of infections and other adverse events in subjects treated with tocilizumab (4).

CASE REPORT

A 53-year-old Japanese man, who had been treated for 4 years with tocilizumab (8 mg/kg every 4 weeks) for rheumatoid arthritis (RA), developed leg ulcers 2 years after starting treatment. A skin biopsy was performed at the time and this showed leukocytoclastic vasculitis that was an extra-articular manifestation of RA. Two years later, he presented with redness around the left leg ulcer associated with inappropriate local treatment for the past 5 days. In addition, extensive haemorrhagic bulla formation and erosions were found around the left leg ulcer. He was admitted to a local hospital and treated with antibiotics for 2 days. Despite this, the redness spread from the left leg ulcer to the mid-calf, and he had difficulty walking due to severe pain.

He was referred to our hospital 24 days after the last tocilizumab administration. He presented with widespread purpura and sclerotic change with normal local temperature, extending from his left toe to mid-

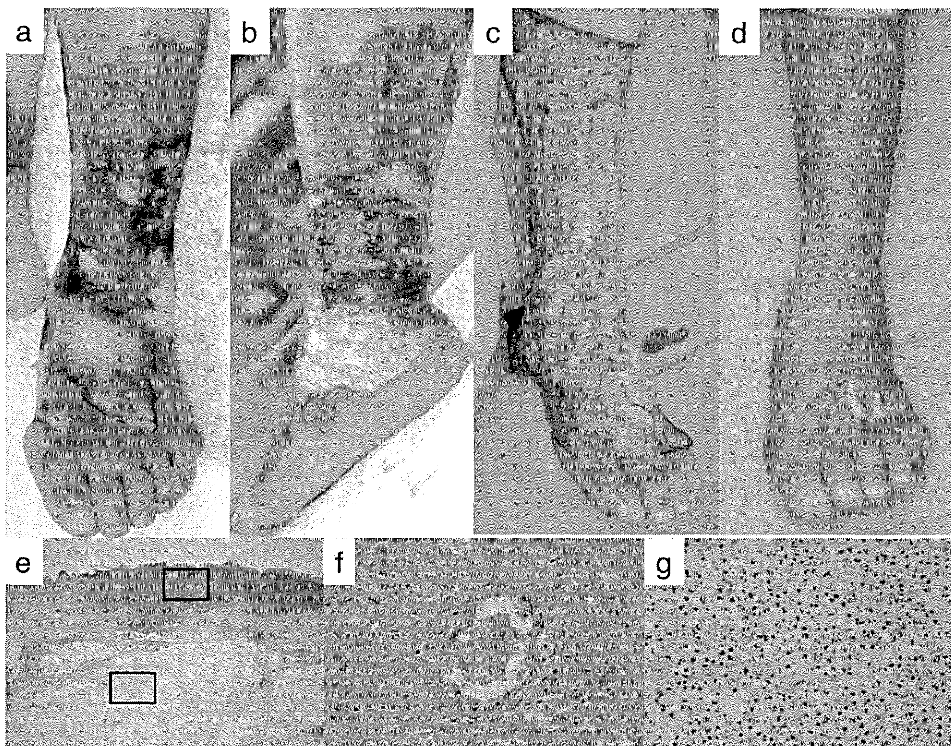

Fig. 1. Haemorrhagic bulla formation and sclerotic change extending from left toe to mid-calf are observed (a). Skin ulcer was observed near the foot joint for 2 years (b). Radical surgical debridement was performed 5 h after admission (c). A split skin graft was applied (d). Histopathological findings from haematoxylin- and eosin-stained tissue sections show presence of thrombi and severe neutrophil infiltration (e–g) (e: × 20; f and g: × 400).

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calf (Fig. 1a, b). His vital signs were as follows: blood pressure, 161/92 mmHg; pulse, 108 beats/min; and body temperature, 36.9°C. Results of laboratory tests were as follows: white blood cell count, 22.1×10^9/l (neutrophils, 89%); haemoglobin, 9.8 g/dl; CRP, 25.3 mg/dl; sodium, 130 mEq/l; creatinine, 0.66 mg/dl; glucose, 154 mg/dl; serum IL-6, 218 pg/ml; and serum vascular endothelial growth factor (VEGF), 572 pg/ml. Computed tomography imaging of the affected regions on the lower left leg showed swelling and contrast enhancement in the subcutaneous tissue. Furthermore, exploratory incision of diseased skin exuded a watery, slightly scented fluid. Microbial culture tests revealed Group A Streptococcus heavy growth. Radical surgical debridement was performed 5 h after admission (Fig. 1c). On the second day of hospitalization, serum IL-6 and VEGF levels had decreased to 57.6 pg/ml and 255 pg/ml, respectively. The patient was treated in the intensive care unit post-surgery with intravenous penicillin G (24×10^6 U/day) and meropenem (3 g/day) and he gradually showed improvement. At day 21 after the initial debridement, a split skin graft was successfully transplanted (Fig. 1d), and he was discharged at day 70.

DISCUSSION

Histopathological findings from necrotic tissue revealed severe neutrophil infiltration, and occlusion of the vascular lumen by thrombi was detected (Fig. 1e-g). This type of occlusion in patients with NF has been previously described (5). In this case, serum VEGF levels dramatically decreased after debridement of necrotic tissue. It is known that VEGF contributes to growth of vessels in inflammatory diseases (6). Thus, we speculate that there is an association between occlusion of the vascular lumen in necrotic tissue and elevated serum VEGF levels in patients with NF.

Lack of constitutional symptoms in the presence of serious infection has been reported in RA patients treated with tocilizumab (7). Mild adverse events may often be overlooked and these may lead to lethal complications. We have identified 2 other published reports that have evaluated NF in patients who were treated with tocilizumab for RA (8, 9). In those cases, it was difficult to make an initial diagnosis of NF because the patients lacked typical laboratory findings, such as elevated CRP and white blood cell count. In the NF case described here, the CRP levels were abnormally high. It is possible that these controversial findings were influenced by the period of time since the final tocilizumab administration. However, all cases reported severe pain, and none of them described local warmth. Appropriate diagnostic methods, including computed tomography imaging, needle aspiration, and exploratory incision, have led to accurate diagnosis, and they can improve the prognosis of patients who develop NF following tocilizumab treatment.

Our experience highlights the fact that increased attention and low investigation threshold should be maintained in patients treated with tocilizumab. Early recognition and treatment of serious infections may improve prognosis in patients treated with biologics.

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Original Investigation

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 10q23.33 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; $P=1.1 \times 10^{-17}$). 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 11 (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 antiepileptic drug and remains the most frequently used first-line antiepileptic drug in hospitalized patients.[1,2] 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 variants of the same mucocutaneous blistering reaction disease and carry high morbidity and mortality (10%-50%).[4-6] DRESS, also known as drug-induced hypersensitivity syndrome, is characterized by generalized maculopapular eruptions, high fever, eosinophilia, atypical lymphocytes, and visceral involvement and has a mortality rate of approximately 10%.[6,7] Phenytoin-related severe cutaneous adverse reactions frequently impair the internal organs, leading to the highest mortality among the different antiepileptic drug-related cutaneous reactions.[5]

ALDEN *algorithm of drug causality for epidermal necrolysis*

DRESS *drug reaction with eosinophilia and systemic symptoms*

GWAS *genome-wide association study*

SJS *Stevens-Johnson syndrome*

SNP *single-nucleotide polymorphism*

TEN *toxic epidermal necrolysis*

The pharmacogenomic basis of phenytoin-related severe cutaneous adverse reactions remains unknown. By candidate gene approach, *HLA-B*15:02* was identified as associated with phenytoin-related severe cutaneous adverse reactions in Asians; however, the strength of the association is much weaker than that found for carbamazepine-related SJS-TEN.[8-12] The US Food and Drug Administration has suggested that physicians should avoid prescribing phenytoin or fosphenytoin as an alternative to carbamazepine in patients who carry *HLA-B*15:02*.[13] To investigate the genetic factors associated with phenytoin-related severe cutaneous adverse reactions, we carried out a genome-wide association study (GWAS) followed by direct sequencing of the associated genes and replication analyses using samples from Taiwan, Japan, and Malaysia.

Methods

Recruitment of Cases and Drug-Tolerant Controls

Patients with phenytoin-related cutaneous adverse reactions were recruited from the Chang Gung Memorial Hospital (CGMH) health system and Taiwan Severe Cutaneous Adverse Reactions Consortium in Taiwan, Hospital Sultanah Aminah Johor Bahru in Malaysia, and centers collaborating with the National Institute of Health Sciences and Osaka University in Japan between 2002 and 2014. All cases were evaluated by at least 2 dermatologists, who reviewed all available photographs, histological data, and clinical information, including type of cutaneous reactions, date of onset, and drug history, dosage, and duration. Phenotypes of severe cutaneous adverse reactions were clinically assessed using the diagnostic criteria established by the Registry of Severe Cutaneous Adverse Reactions Consortium, which maintains a multinational registry of severe cutaneous adverse reactions cases reported by physicians from many countries (Austria, France, Germany, Israel, Italy, the Netherlands, Spain, South Africa, Taiwan, the United Kingdom, and Vietnam, etc).[4,7] 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

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containing the *CYP2C* region. We calculated the D' and r^2 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 Axsym 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 $P=5.9\times10^{-8}$ 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 τ^2 and I^2. 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.

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 ($P<5.9\times10^{-8}$) 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), *CYP2C19* (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 (*CYP2C19*1C*; p.V331I), showing statistically significant association with phenytoin-related severe cutaneous adverse reactions (Table 2 and

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Table 1. Demographic Data and Clinical Features of Patients With Phenytoin-Related Cutaneous Adverse Reactions and Phenytoin-Tolerant Controls Enrolled in Taiwan

	Cases of Phenytoin-Related Cutaneous Adverse Reactions (n = 168)						
	Severe Cutaneous Adverse Reactions (n = 90)						
Characteristics	SJS (n = 39)	SJS-TEN Overlapping (n = 3)	TEN (n = 6)	DRESS (n = 42)	Total (n = 90)	MPE (n = 78)	Phenytoin-Tolerant Controls (n = 130)
Age, y							
Mean (SD)	56 (17)	63 (3.5)	64 (16)	57 (21)	57 (17)	52 (21)	41 (14)
Median (range)	59 (19-77)	61 (61-67)	68 (37-77)	59 (13-91)	61 (13-91)	54 (1-88)	39 (15-79)
Sex, No. (%)							
Male	15 (39)	1 (33)	3 (50)	18 (43)	37 (41)	39 (50)	82 (63)
Female	24 (61)	2 (67)	3 (50)	24 (57)	53 (59)	39 (50)	48 (37)
Phenytoin exposure							
Dosage, mean (95% CI) [range], mg/d	309 (268-343) [100-750]	300 (300-300) [300-300]	325 (245-405) [300-400]	317 (289-339) [300-750]	314 (292-330) [100-750]	312 (287-338) [100-800]	323 (309-337) [100-600]
Duration, mean (range), d	23 (2-58)[a]	21 (10-31)	24 (12-63)	30 (7-59)	27 (2-63)[a]	23 (2-58)	89 mo (3-303 mo)
Deceased cases, No. (%)	2 (5.1)	2 (67)	5 (83)	4 (9.5)	13 (14)	0	0
Internal organ involvement							
Hepatitis, GPT, IU/L, No. (%)[b]							
<100	18 (46)	3 (100)	3 (50)	16 (38)	40 (44)	NA	NA
100-500	15 (39)	0	3 (50)	18 (43)	36 (40)	NA	NA
501-1000	5 (13)	0	0	4 (9.5)	9 (10)	NA	NA
>1000	1 (3.1)	0	0	4 (9.5)	5 (5.5)	NA	NA
Acute renal failure[c]	2 (6.3)	2 (67)	1 (17)	6 (14)	11 (12)	NA	NA
Hematologic abnormalities							
Eosinophilia, absolute eosinophil counts/μL, No. (%)							
<700	20 (51)	2 (67)	4 (67)	13 (31)	39 (43)	NA	NA
700-1499	15 (39)	1 (33)	1 (17)	17 (41)	34 (38)	NA	NA
≥1500	4 (10)	0	1 (17)	12 (29)	17 (19)	NA	NA
Atypical lymphocytosis	12 (31)	1(33)	4 (67)	22 (52)	39 (43)	NA	NA
Mucosal involvement, No. (%)							
Oral	39 (100)	3 (100)	6 (100)	16 (38)	64 (71)	NA	NA
Eyes	19 (49)	2 (67)	5 (83)	3 (7.1)	29 (32)	NA	NA
Genital	12 (31)	2 (67)	4 (67)	1 (2.4)	19 (21)	NA	NA

Abbreviations: DRESS, drug reaction with eosinophilia and systemic symptoms; GPT, glutamic pyruvic transaminase; MPE, maculopapular exanthema; NA, not applicable; SJS, Stevens-Johnson syndrome; TEN, toxic epidermal necrolysis.

[a] A duration of 2 days was observed in a patient who received intravenous phenytoin infusion.

[b] Values of glutamic-pyruvic transaminase were 2 times greater than normal.

[c] The creatinine value was 1.5-fold higher than the normal value range (0.4-1.5 mg/dL) after drug intake.

eTable 2 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

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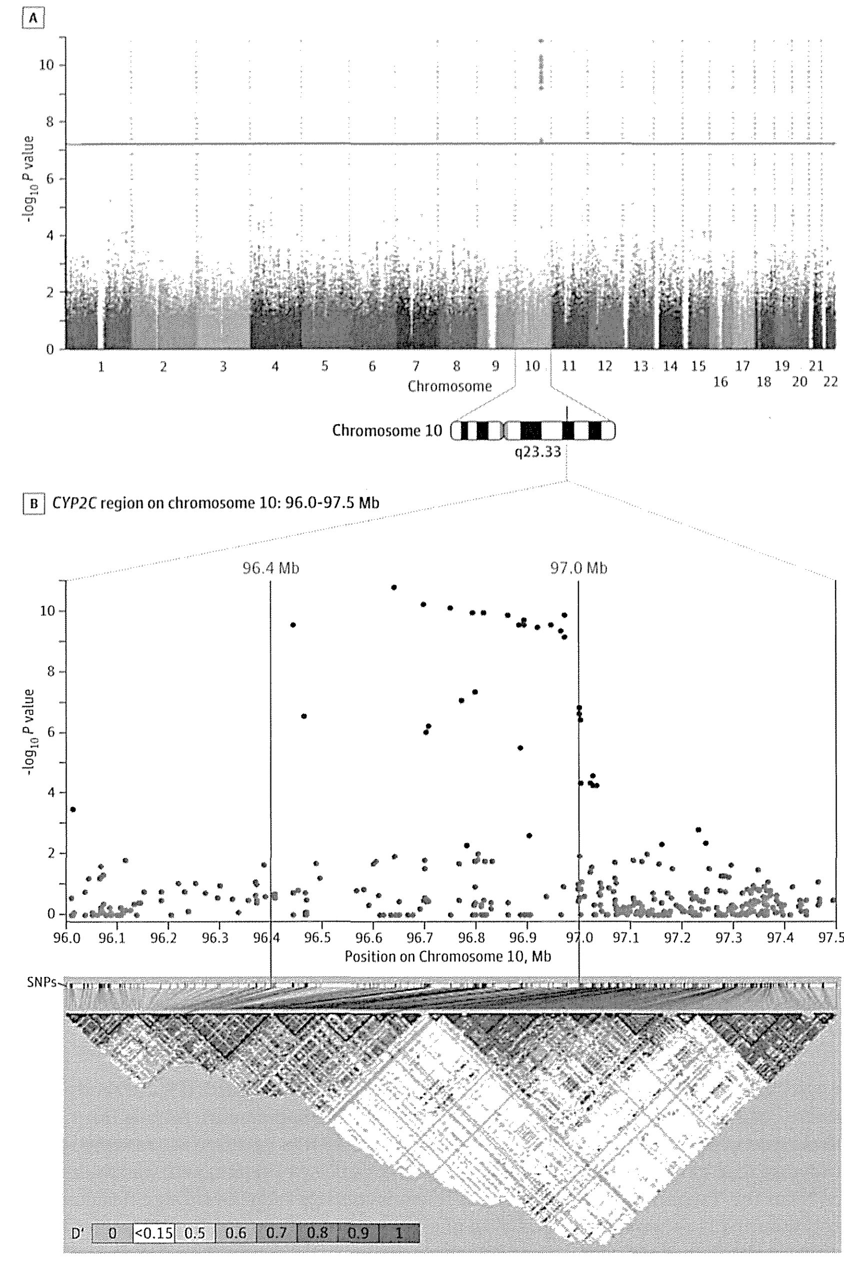
Figure 1. Genome-Wide Association Scan and Linkage Disequilibrium Map for the *CYP2C* Region Associated With Phenytoin-Related Severe Cutaneous Adverse Reactions



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 $P = 5.9 \times 10^{-8}$, 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*, *CYP2C8*, and *C10orf129* 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).

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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Table 2. Ten Significant SNPs Associated With Phenytoin-Related Severe Cutaneous Adverse Reactions in the GWAS Discovery, Direct Sequencing, Replication, and Combined Samples

				GWAS Discovery[b]				Replication Analysis[c]				Combination[d]			
				MAF				MAF						P Value	
SNP	Position on Chromosome 10 (bp)[a]	Nearby Gene (Location)[a]	Minor allele	Cases	Controls	P Value[e]	OR (95% CI)	Cases	Controls	P Value[e]	OR (95% CI)	P Value[e]	OR (95% CI)	HET	HWE
rs17110192	10q23.33 (96441927)	*CYP2C18* (5'UTR)	C	0.2	0.026	1.5×10^{-11}	9.6 (5.1-18)	0.18	0.019	9.0×10^{-6}	11.5 (3.9-34)	5.5×10^{-16}	9.8 (5.8-17)	.78	.15
rs3758581 (*CYP2C19*1C*)	10q23.33 (96602623)	*CYP2C19* (exon 7)	A	0.21	0.03	3.4×10^{-11}	8.4 (4.6-15)	0.18	0.023	2.2×10^{-5}	9.5 (3.4-27)	3.5×10^{-15}	8.5 (5.1-14)	.79	.30
rs17110321	10q23.33 (96639896)	*CYP2C9*, *CYP2C19*	G	0.2	0.026	1.5×10^{-11}	9.6 (5.1-18)	0.18	0.012	1.0×10^{-6}	19.2 (5.2-71)	1.3×10^{-16}	11 (6.2-18)	.34	.15
rs9332093	10q23.33 (96696555)	*CYP2C9* (5'UTR)	G	0.2	0.026	1.5×10^{-11}	9.6 (5.1-18)	0.18	0.015	3.3×10^{-6}	14.4 (4.4-47)	2.7×10^{-16}	10 (6.0-18)	.55	.15
rs1057910 (*CYP2C9*3*)	10q23.33 (96741053)	*CYP2C9* (exon 7)	C	0.21	0.024	1.5×10^{-12}	11 (5.7-20)	0.18	0.012	1.0×10^{-6}	19.2 (5.2-71)	1.1×10^{-17}	12 (6.6-20)	.42	.61
rs9332245	10q23.33 (96749181)	*CYP2C9* (3'UTR)	A	0.2	0.026	1.5×10^{-11}	9.6 (5.1-18)	0.18	0.019	9.0×10^{-6}	11.5 (3.9-34)	5.5×10^{-16}	9.8 (5.8-17)	.78	.15
rs1592037	10q23.33 (96792328)	*CYP2C8* (3'UTR)	A	0.21	0.033	1.0×10^{-10}	7.8 (4.3-14)	0.18	0.035	1.8×10^{-4}	6.3 (2.5-16)	6.9×10^{-14}	7.3 (4.4-12)	.70	.39
rs6583967	10q23.33 (96814475)	*CYP2C8* (Intron)	C	0.21	0.033	1.0×10^{-10}	7.8 (4.3-14)	0.2	0.027	1.2×10^{-5}	9.0 (3.4-24)	5.1×10^{-15}	8.0 (4.9-13)	.79	.39
rs10882551	10q23.33 (96905783)	*CYP2C8* (5'UTR)	T	0.2	0.033	4.6×10^{-10}	7.4 (4.1-13)	0.2	0.019	2.0×10^{-6}	12.8 (4.3-38)	6.5×10^{-15}	8.2 (5.0-14)	.38	.39
rs12262878	10q23.33 (96971504)	*C10orf129* (Intron)	C	0.2	0.03	1.6×10^{-10}	8.0 (4.4-15)	0.18	0.027	4.8×10^{-5}	8.1 (3.0-22)	2.9×10^{-14}	7.9 (4.8-13)	.98	.30

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, untranslational region.

[a] The genomic coordinates are based on NCBI Human Genome Build 37.5. Gene ID: *CYP2C18*: NCBI Entrez gene 1562; *CYP2C19*: NCBI Entrez gene 1557; *CYP2C9*: NCBI Entrez gene 1559; *CYP2C8*: NCBI Entrez gene 1558; and *C10orf129*: NCBI Entrez gene 142827.

[b] Sixty cases of severe cutaneous adverse reactions vs 412 controls from the general population.

[c] Thirty cases of severe cutaneous adverse reactions vs 130 phenytoin-tolerant controls.

[d] Ninety cases of severe cutaneous adverse reactions vs 542 controls.

[e] *P* values were calculated by Fisher exact test for the risk allele. All SNPs were discovered by the GWAS except rs3758581 and rs1057910, which were identified by direct sequencing on the associated genes.

samples from 9 patients with SJS-TEN and 2869 population controls from Japan and 6 severe cutaneous adverse reactions cases and 374 population controls from Malaysia.[17] Demographic and clinical data for patients with phenytoin-related severe cutaneous adverse reactions from Japan and Malaysia are shown in eTable 8 in the Supplement. Allele frequencies of *CYP2C9*3* were 17% to 22% in samples from patients with phenytoin-related severe cutaneous adverse reactions but only 2.7% to 2.8% in samples from the population controls of Japan and Malaysia (eTable 9 in the Supplement). *CYP2C9*3* showed statistically significant association with phenytoin-related severe cutaneous adverse reactions in both Japanese (OR, 10; 95% CI, 3.4-32; $P = 1.2 \times 10^{-3}$) and Malaysians (OR, 6.9; 95% CI, 1.4-34; $P = .048$) (eTable 9). We further analyzed the association between *CYP2C9*3* and phenytoin-related severe cutaneous adverse reactions by meta-analysis using a random-effects model and classified cases and controls according to their phenotype (SJS-TEN or DRESS) and ethnicity (Taiwanese, Japanese, or Malaysian) (**Figure 3**). The results of the meta-analysis showed a pooled OR of 12 (95% CI, 6.4-22; $z = 7.82$; $P < .00001$) for a *CYP2C9*3* association with phenytoin-related SJS-TEN, a pooled OR of 9.2 (95% CI, 4.3-20; $z = 5.70$; $P < .00001$) for a *CYP2C9*3* association with phenytoin-related DRESS, and an overall OR of 11 (95% CI, 6.2-18; $z = 8.58$; $P < .00001$) for a *CYP2C9*3* association with phenytoin-related severe cutaneous adverse reactions in Asians (Figure 3).

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; $P = 7.0 \times 10^{-4}$; $P = .025$ 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 and 40 patients with severe cutaneous adverse reactions was labeled as day 0 (eFigure 6, B-D). Nine samples from patients with severe cutaneous adverse reac-

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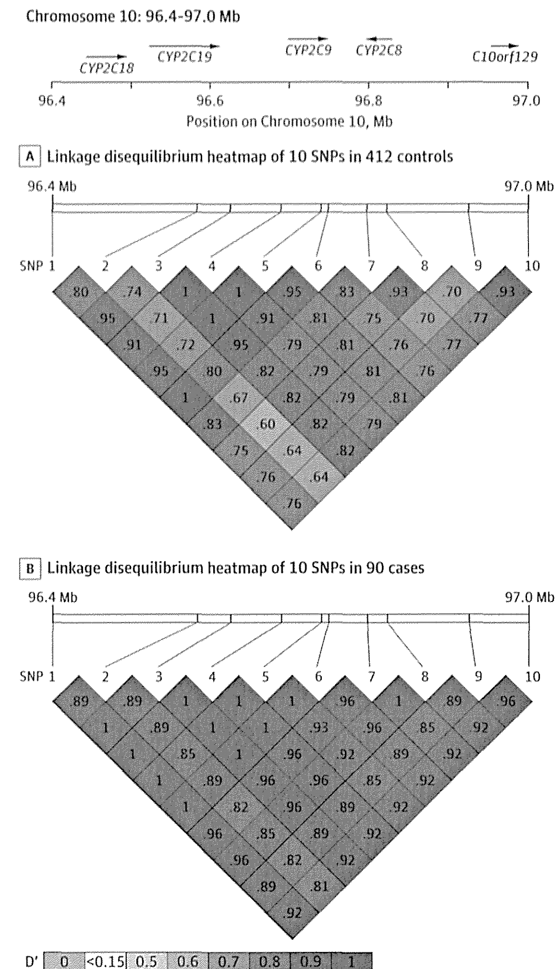
tions were obtained before drug withdrawal because these patients were hospitalized and received phenytoin for seizure prophylaxis. Before drug withdrawal, plasma concentrations of phenytoin were significantly higher in patients with SJS-TEN (mean, 34 μg/mL; 95% CI, 1.8-66 μg/mL) compared with the phenytoin-tolerant 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*3* 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.

Figure 2. Linkage Disequilibrium Heat Maps for the *CYP2C* Region Associated With Phenytoin-Related Severe Cutaneous Adverse Reactions



The linkage disequilibrium heat maps are drawn based on pairwise D' values of the 10 risk single-nucleotide polymorphisms using the data of 412 controls (A) and 90 cases of phenytoin-related severe cutaneous adverse reactions (B).

Discussion

Phenytoin has a narrow therapeutic range (10-20 μg/mL) and nonlinear pharmacokinetics and is metabolized to inactive hydroxyphenytoin, 5-(4'-hydroxyphenyl)-5-phenylhydantoin (p-HPPH), primarily (90%) by the cytochrome P450 (CYP) 2C9 enzyme.[19] Formation of p-HPPH is thought to proceed via a reactive arene oxide intermediate, which has been proposed for the induction of phenytoin hypersensitivity.[19,20] In this study, we report *CYP2C* variants, including *CYP2C9*3*, known to cause 93% to 95% reduction in phenytoin clearance,[21-24] 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 strength of 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.[25-27] 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.[22,23] The SNP rs1057910 forms *CYP2C9*3* and part of *CYP2C9*18*. Another risk SNP, rs3758581, present on the *CYP2C19*1B* and *CYP2C19*1C* normal haplotypes, is a missense mutation yet has no obvious effects on CYP2C19 activity or drug metabolism.[24,28,29] 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%).[28-30] *CYP2C9*3* was reported to be associated with phenytoin maculopapular exanthema (P = .007) in Koreans.[31] A GWAS using samples from 40 cases

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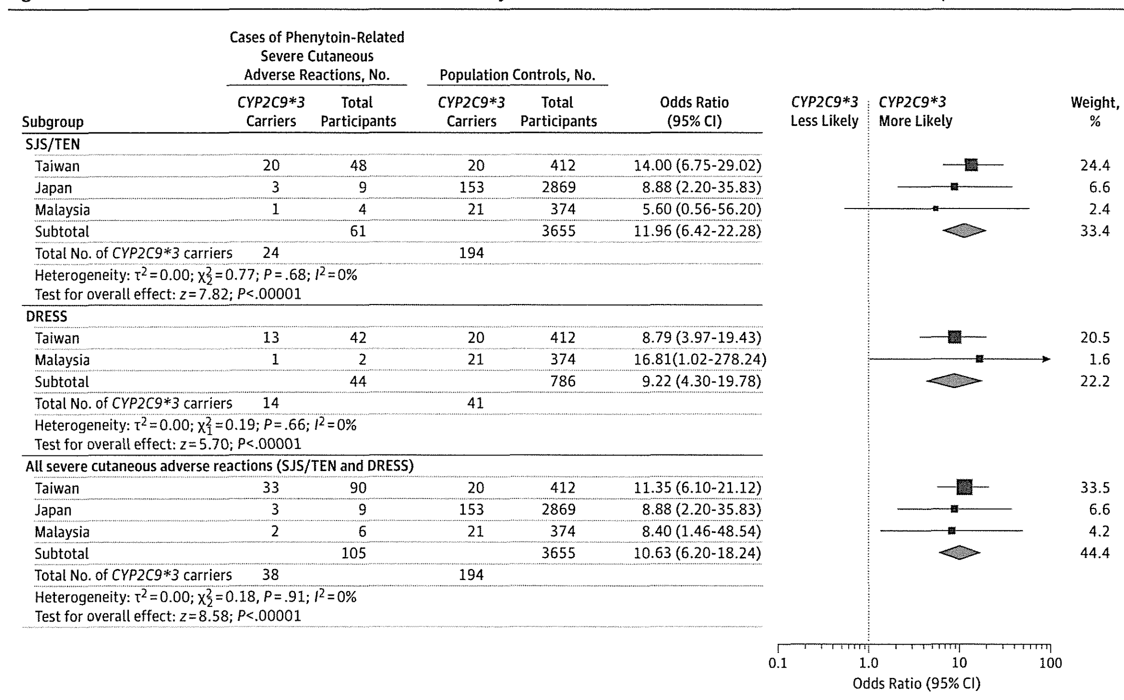
Figure 3. Distribution of the *CYP2C9*3* Variant in Cases With Phenytoin-Related Severe Cutaneous Adverse Reactions and Population Controls



Subgroup	Cases of Phenytoin-Related Severe Cutaneous Adverse Reactions, No. *CYP2C9*3* Carriers	Total Participants	Population Controls, No. *CYP2C9*3* Carriers	Total Participants	Odds Ratio (95% CI)	Weight, %
SJS/TEN						
Taiwan	20	48	20	412	14.00 (6.75-29.02)	24.4
Japan	3	9	153	2869	8.88 (2.20-35.83)	6.6
Malaysia	1	4	21	374	5.60 (0.56-56.20)	2.4
Subtotal		61		3655	11.96 (6.42-22.28)	33.4
Total No. of *CYP2C9*3* carriers	24		194			
Heterogeneity: $\tau^2=0.00$; $\chi^2_2=0.77$; $P=.68$; $I^2=0\%$						
Test for overall effect: $z=7.82$; $P<.00001$						
DRESS						
Taiwan	13	42	20	412	8.79 (3.97-19.43)	20.5
Malaysia	1	2	21	374	16.81(1.02-278.24)	1.6
Subtotal		44		786	9.22 (4.30-19.78)	22.2
Total No. of *CYP2C9*3* carriers	14		41			
Heterogeneity: $\tau^2=0.00$; $\chi^2_1=0.19$; $P=.66$; $I^2=0\%$						
Test for overall effect: $z=5.70$; $P<.00001$						
All severe cutaneous adverse reactions (SJS/TEN and DRESS)						
Taiwan	33	90	20	412	11.35 (6.10-21.12)	33.5
Japan	3	9	153	2869	8.88 (2.20-35.83)	6.6
Malaysia	2	6	21	374	8.40 (1.46-48.54)	4.2
Subtotal		105		3655	10.63 (6.20-18.24)	44.4
Total No. of *CYP2C9*3* carriers	38		194			
Heterogeneity: $\tau^2=0.00$; $\chi^2_2=0.18$, $P=.91$; $I^2=0\%$						
Test for overall effect: $z=8.58$; $P<.00001$						

Patients with phenytoin-related severe cutaneous adverse reactions were recruited at the Chang Gung Memorial Hospital health system and the Taiwan Severe Cutaneous Adverse Reaction Consortium in Taiwan, Hospital Sultanah Aminah Johor Bahru in Malaysia, and centers collaborating with the National Institute of Health Sciences and Osaka University in Japan. Study weighting (indicated by size of data markers) refers to the proportion of participants who were recruited from each study. The τ^2 and I^2 represent measures of heterogeneity. Diamonds represent pooled odds ratios (Mantel-Haenszel method, random effects) and error bars indicate 95% CIs. DRESS indicates drug reaction with eosinophilia and systemic symptoms; SJS, Stevens-Johnson syndrome; TEN, toxic epidermal necrolysis.

with maculopapular exanthema and 4 cases with severe cutaneous adverse reactions caused by phenytoin and 1296 controls from a British population[32] 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.[33-38] 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.[39,40] 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.

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