SHORT COMMUNICATION

Up-regulation of Human Herpesvirus 6B-derived microRNAs in the Serum of Patients with Drug-induced Hypersensitivity Syndrome/Drug Reaction with Eosinophilia and Systemic Symptoms

Kazuya MIYASHITA, Fumi MIYAGAWA, Yuki NAKAMURA, Rie OMMORI, Hiroaki AZUKIZAWA and Hideo ASADA*
*Department of Dermatology, Nara Medical University School of Medicine, 840 Shijo-cho, Kashihara, Nara 634-8522, Japan. *E-mail: asadah@naramed-u.ac.jp*
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Drug-induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms (DIHS/DRESS) is a life-threatening multi-organ hypersensitivity reaction. Reactivation of human herpesvirus 6B (HHV-6B), which typically occurs 2–3 weeks after its onset, has been implicated in DIHS/DRESS (1). Reactivation of HHV-6 has been reported to correlate with flaring of symptoms such as fever and hepatitis (2) and renal failure (3) in patients with DIHS/DRESS, indicating that virus reactivation could contribute to some symptoms or complications in DIHS/DRESS. However, it has also been reported that reactivation of HHV-6 could be merely a result of a strong drug-specific immune response and not contribute to DRESS symptoms and severity (4).

MicroRNAs (miRNAs) play important roles in biological processes such as immune responses and cell differentiation. Herpesviruses express their own miRNAs and may regulate key viral genes (5). HHV-6A encodes miR-U86 that regulates viral lytic replication (6), while HHV-6B encodes at least 4 miRNAs: hhv6b-miR-Ro6-1, -2, -3 and -4 (7). However, the precise roles of these 4 miRNAs in the regulation of HHV-6B latency and reactivation remain largely unknown. Moreover, the roles of individual miRNAs in DIHS/DRESS have not yet been elucidated. The present study investigated the expression levels of the 4 HHV-6B miRNAs in the serum of patients with DIHS/DRESS during the acute and subacute stages.

MATERIALS AND METHODS (see Appendix S1[1])

RESULTS

The maximum levels of hhv6b-miR-Ro6-1, -2, -3, and -4 in serum were significantly higher in patients with DIHS/DRESS than in those with MPE and healthy controls ($p < 0.05$, respectively) (**Fig. 1**a).

The time course of HHV-6B miRNA expression was examined in the serum of patients with DIHS/DRESS. In case 1, HHV-6B reactivation was confirmed by detecting HHV-6B DNA in peripheral blood mononuclear cells (PBMCs) on day 25 after onset. The expression of hhv6b-miR-Ro6-2 in serum was detected on day 19, while hhv6b-miR-Ro6-4 and -1 were detected on days 25 and 33, respectively (Fig. S1a[1]).

In case 2, HHV-6B reactivation was detected on day 16 after onset. Hhv6b-miR-Ro6-2 was expressed on day 10, while hhv6b-miR-Ro6-3 and -1 were expressed on the same day as HHV-6B DNA was detected (Fig. S1b[1]).

[1]https://www.medicaljournals.se/acta/content/abstract/10.2340/00015555-2925

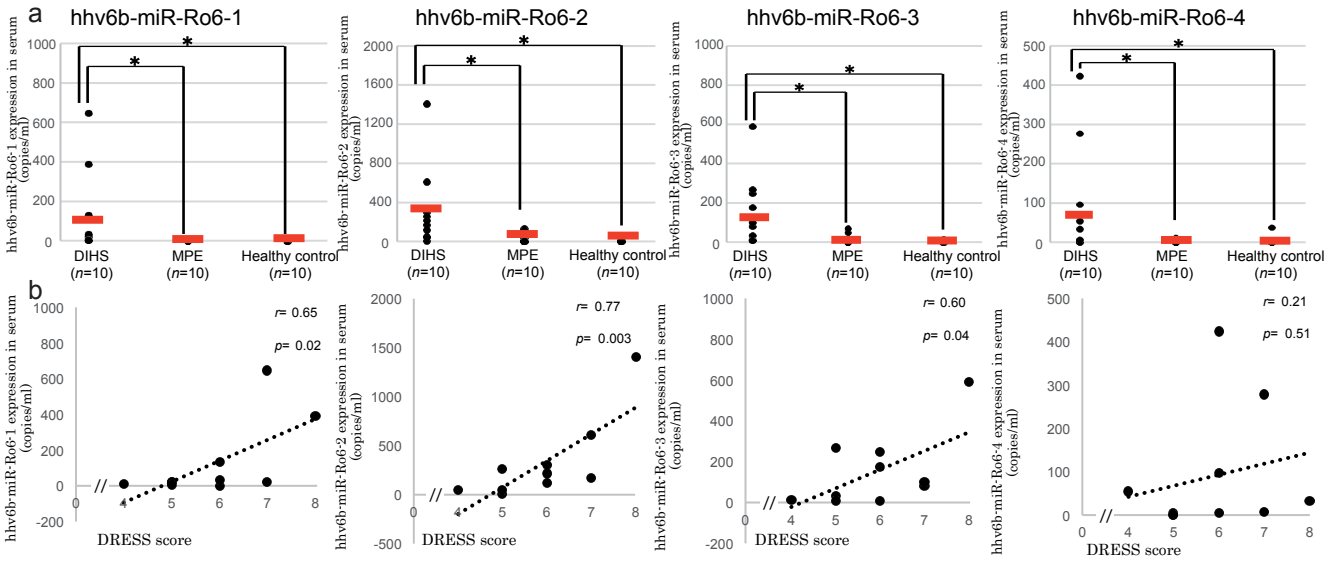

Fig. 1. (a) **Up-regulation of human herpesvirus 6B (HHV-6B)-derived miRNAs in the serum of patients with drug-induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms (DIHS/DRESS).** The maximum levels of HHV6b-miR-Ro6-1, -2, -3, and -4 in serum were significantly higher in patients with DIHS/DRESS than in those with maculo-papular eruption (MPE) and healthy controls. *$p < 0.05$. (b) **Correlation between DRESS scores and HHV-6B miRNAs in the serum of patients with DIHS/DRESS.** DRESS scores correlated with the serum levels of hhv6b-miR-Ro6-1, -2, and -3, respectively.

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In case 6, the expression of HHV-6B DNA and hhv6b-miR-Ro6-2 and -3 was detectable on day 9 after onset, while hhv6b-miR-Ro6-4 was detected on day 21 following hhv6b-miR-Ro6-2 expression (Fig. S1c[1]).

It was then investigated whether HHV-6B miRNA levels correlated with clinical symptoms and laboratory data. The RegiSCAR scoring system (DRESS score) was used to evaluate the severity of clinical symptoms in patients with DIHS/DRESS. Ten patients with DIHS/DRESS (4 men and 6 women) were graded according to DRESS scores as "probable" ($n=4$) or "definite" ($n=6$) (Table SI[1]). As shown in Fig. 1b, DRESS scores correlated with the serum levels of hhv6b-miR-Ro6-1 ($r=0.65$, $p=0.02$), hhv6b-miR-Ro6-2 ($r=0.77$, $p=0.003$), and hhv6b-miR-Ro6-3 ($r=0.60$, $p=0.04$). DRESS scores were weakly associated with the serum levels of hhv6b-miR-Ro6-4 ($r=0.21$, $p=0.51$).

Relationships between the serum levels of HHV-6B miRNAs and each variable in the clinical and laboratory data were examined. The expression levels of HHV6B-derived miRNAs were not associated with liver function test results, eosinophil counts, the percentage of atypical lymphocytes, cervical lymphadenopathy, or the HHV-6B DNA levels of PBMC (data not shown). However, as shown in Fig. S2[1], the duration of fever (>38.0°C) correlated with serum levels of hhv6b-miR-Ro6-2 ($r=0.72$, $p=0.01$) and hhv6b-miR-Ro6-3 ($r=0.69$, $p=0.01$). The duration of fever was weakly associated with the serum levels of hhv6b-miR-Ro6-1 ($r=0.30$, $p=0.34$), but not with those of hhv6b-miR-Ro6-4 ($r=0.005$, $p=0.99$).

Serum levels of hhv6b-miR-Ro6-2 were associated with the severity of skin lesions (Table SII[1]). When the expression levels of hhv6b-miR-Ro6-2 in DIHS/DRESS patients were listed in descending order, the first 8 patients with higher levels of hhv6b-miR-Ro6-2 had erythroderma, while the last 2 patients with lower levels of hhv6b-miR-Ro6-2 had diffuse MPE. hhv6b-miR-Ro6-2 may reflect the type of skin eruption. Neither Hhv6b-miR-Ro6-1, -3, nor -4 were associated with the type of skin eruption.

DISCUSSION

HHV-6B encodes at least 4 miRNAs: hhv6b-miR-Ro6-1, -2, -3 and -4. These 4 HHV-6B-derived miRNAs were identified in Sup-T-1 cells infected with HHV-6B using a deep sequencing approach and expressed during lytic infection (7). Hhv6b-miR-Ro6-2 and -3 are detectable very early after infection and are encoded antisense to the immediate-early (IE) genes (8). Hhv6b-miR-Ro6-1 is detected 2 days after the expression of hhv6b-miR-Ro6-2 and -3, and is encoded antisense to IE (9) or early genes (8). Hhv6b-miR-Ro6-4 is detected 4 days after HHV-6B infection (7). As shown in Fig. S1[1], our results showed that the serum levels of hhv6b-miR-Ro6-2 were increased before or at the same time as the detection of HHV-6B DNA, while those of hhv6b-miR-Ro6-1 and/or -4 were significantly increased a few weeks later than hhv6b-miR-Ro6-2 expression in some patients with DIHS/DRESS. The kinetics of the emergence of hhv6b-miR-Ro6-2, -1, and -4 in DIHS/DRESS in the present study were mostly consistent with the *in vitro* findings reported by Tuddenham et al. (7). These results suggest that hhv6b-miR-Ro6-2 and hhv6b-miR-Ro6-1/-4 have distinct functions in the regulation of HHV-6B reactivation.

We also demonstrated that the expression of hhv6b-miR-Ro6-1, -2, and -3 was associated with DRESS scores, while that of hhv6b-miR-Ro6-2 and -3 was associated with the duration of fever. These results suggest that the serum levels of HHV-6B miRNAs may be useful indicators of the severity of DIHS/DRESS.

In conclusion, the detection of the miRNAs of HHV-6B in DIHS/DRESS may reflect the reactivation of HHV-6B, and hhv6b-miR-Ro6-2 may be an early and specific biomarker for predicting the reactivation of HHV-6B. We consider these results, which were obtained by identifying a number of differentially expressed HHV-6B miRNAs in the course of DIHS/DRESS, to provide novel insights into the molecular pathogenesis of DIHS/DRESS.

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The authors have no conflicts of interest to declare.

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Research letter

Serum thymus and activation-regulated chemokine is associated with the severity of drug reaction with eosinophilia and systemic symptoms/drug-induced hypersensitivity syndrome

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Dear Editor, Drug reaction with eosinophilia and systemic symptoms (DRESS)/drug-induced hypersensitivity syndrome (DIHS) is a severe adverse drug-induced reaction with reactivation of human herpesvirus (HHV)-6.[1–3] We previously reported that serum thymus and activation-regulated chemokine (TARC) levels were markedly increased in patients with DIHS and suggested that TARC is a useful diagnostic marker of DIHS in the early stage.[4,5] In this study, we determined whether serum TARC levels correlate with the severity of clinical symptoms and laboratory data in patients with DRESS/DIHS.

We evaluated 16 patients with DRESS/DIHS (eight male and eight female, median age 44 years and 68·5 years, respectively) for their clinical symptoms and laboratory data. Recorded data included copy numbers of HHV-6 and human cytomegalovirus (CMV) DNA in peripheral blood mononuclear cells, serum cytokines and soluble interleukin-2 receptor (sIL-2R), and serum TARC. This was carried out under the approval of the ethics committee at Nara Medical University. Serum TARC levels increased in the acute stage and decreased upon remission of the skin eruption. We evaluated the peak levels of serum TARC in the acute stage.

We first evaluated the association of clinical symptoms with TARC using a severity score of skin and mucosal lesions that we developed. The severity of skin and mucosal lesions and the duration of fever (≥ 38 °C) showed positive correlations with serum TARC levels (Fig. 1a, b). All 11 patients with a high level of serum TARC (≥ 10 000 pg mL^{-1}) developed erythroderma, and three of five patients with a lower TARC level (< 10 000 pg mL^{-1}) developed erythroderma. The serum TARC levels also correlated with the DRESS score ($r = 0·35$, $P = 0·18$), as previously reported.[4,5] Moreover, the patient with the highest serum TARC level (105 300 pg mL^{-1}) died of renal failure, suggesting that TARC is related to severe complications.[6]

We next determined the correlations of complete blood count and blood biochemistry with TARC. The percentage of atypical lymphocytes, and alanine transaminase and creatinine levels were positively correlated with serum TARC levels (Fig. 1c, d; for ALT: $r = 0·45$, $P = 0·08$). In contrast, platelet counts had a negative correlation with serum TARC levels (Fig. 1e). There was no significant relationship between TARC and eosinophil counts. These data also suggest that TARC may reflect the severity of the disease.

We then investigated whether serum TARC levels correlated with the levels of HHV-6 and CMV DNA. HHV-6 and CMV DNA in the peripheral blood were assessed by real-time quantitative polymerase chain reaction as previously reported.[7] The maximum copy numbers of HHV-6 and CMV DNA during the clinical course had positive correlations with TARC levels (Fig. 1f; for CMV: $r = 0·46$, $P = 0·18$). These data suggest that TARC may be associated with the extent of reactivation of HHV-6 and CMV. Tohyama *et al.* reported that HHV-6 reactivation is involved in the flaring, such as fever and hepatitis, and severity of DIHS.[3] Their results are in accordance with our findings that serum TARC levels correlate with the clinical severity and HHV-6 reactivation in patients with DRESS/DIHS. TARC may influence the pathological condition of DRESS/DIHS via HHV-6 reactivation. The correlation of the extent of CMV reactivation with TARC suggested that CMV reactivation should be carefully monitored in patients with high levels of TARC.

Finally, we investigated the association of serum cytokines and sIL-2R with TARC. We previously reported that T helper (Th)2-associated chemokines (TARC and macrophage-derived chemokine) were markedly upregulated in DRESS/DIHS, while Th1-associated chemokines (interferon-inducible protein 10) and monokine induced by interferon-γ predominated in Stevens–Johnson syndrome/toxic epidermal necrolysis.[8] We therefore examined the Th2 cytokines IL-10, IL-5 and IL-4. The levels of IL-10 and IL-5 correlated with TARC (Fig. 1g; for IL-5: $r = 0·47$, $P = 0·07$), but IL-4 had no correlation. These results suggest that serum TARC may selectively induce certain Th2 cytokines. In contrast, the Th1 cytokine interferon-γ showed no correlation with serum TARC. The levels of sIL-2R showed a correlation with serum TARC levels (Fig. 1h). The results for sIL-2R and atypical lymphocytes, together with those for Th2 cytokines, might suggest that Th2 cell activation is involved in DRESS/DIHS, as sIL-2R and atypical lymphocytes are related to T-cell activation.

In conclusion, the serum TARC levels in DRESS/DIHS were correlated with the severity of skin and mucosal lesions; fever; dysfunction of liver and kidney; levels of HHV-6 and CMV DNA; and IL-5, IL-10 and sIL-2R. Our results suggest that TARC might be not only a diagnostic marker but also a useful

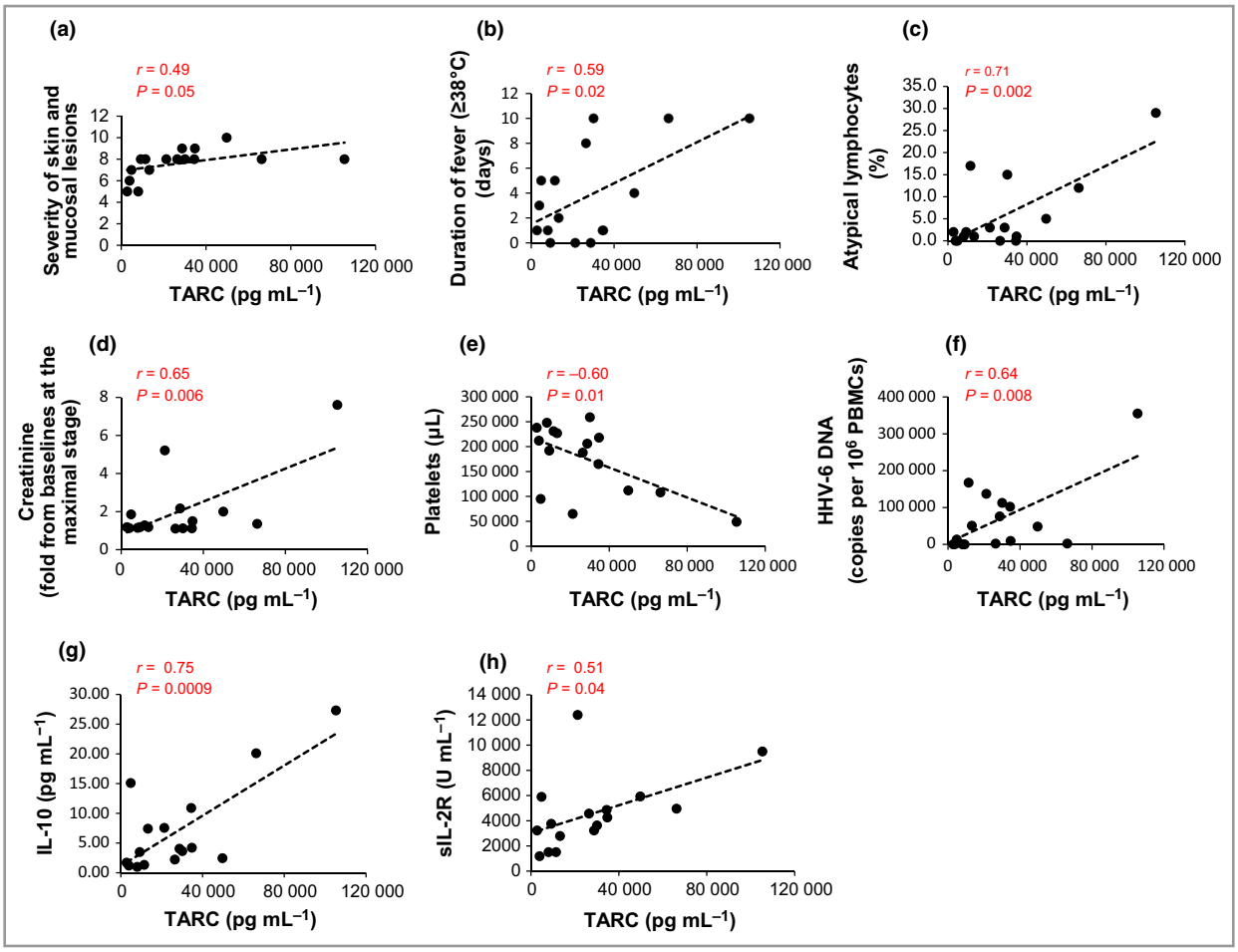

Fig 1. (a) Correlation between the severity of skin and mucosal lesions and serum thymus and activation-regulated chemokine (TARC) levels. The severity score was calculated as the sum of each of the following scores at the peak of disease activity: the extent of erythema (score 1–3); the presence or absence of facial oedema (score 0, 1), purpura (score 0, 1); the number of pustules or scales (score 0–3) and the extent of mucosal lesions (score 0–2). (b–h) Correlations between serum TARC levels and (b) the duration of fever (≥ 38 °C); (c) the percentage of atypical lymphocytes; (d) elevated creatinine levels; (e) blood platelet counts; (f) the peaks of human herpesvirus (HHV)-6 DNA copy level in the acute phase; (g) interleukin (IL)-10 and (h) soluble IL-2 receptor (sIL-2R). PBMC, peripheral blood mononuclear cell.

marker for assessing the clinical and immunological condition of patients.

Department of Dermatology, Nara Medical University School of Medicine, 840 Shijo-cho, Kashihara, Nara 634-8522, Japan
Correspondence: Hideo Asada.
E-mail: asadah@naramed-u.ac.jp

Y. Nakamura-Nishimura
F. Miyagawa
K. Miyashita
R. Ommori
H. Azukizawa
H. Asada

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Conflicts of interest: none to declare.

Research letter

Epidermal growth factor receptor (EGFR) inhibitory monoclonal antibodies and EGFR tyrosine kinase inhibitors have distinct effects on the keratinocyte innate immune response

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Dear Editor, Epidermal growth factor receptor inhibitors (EGFRIs) are a well-established targeted therapy for several cancers. Two categories of EGFRIs are known, EGFR tyrosine kinase inhibitors (TKIs) and EGFR monoclonal antibodies (mAbs). These EGFRIs frequently cause cutaneous adverse effects, such as papulopustular eruptions, xerosis and chronic paronychia. These cutaneous toxicities can result in reduction or even cessation of anti-EGFR therapy and have been shown to compromise patients' quality of life.

We previously reported that EGFR TKIs suppressed the expression of human β-defensins (hBDs) induced by the secreted products of *Staphylococcus epidermidis*, but not by those secreted by *Staphylococcus aureus*.[1] Other groups also reported that

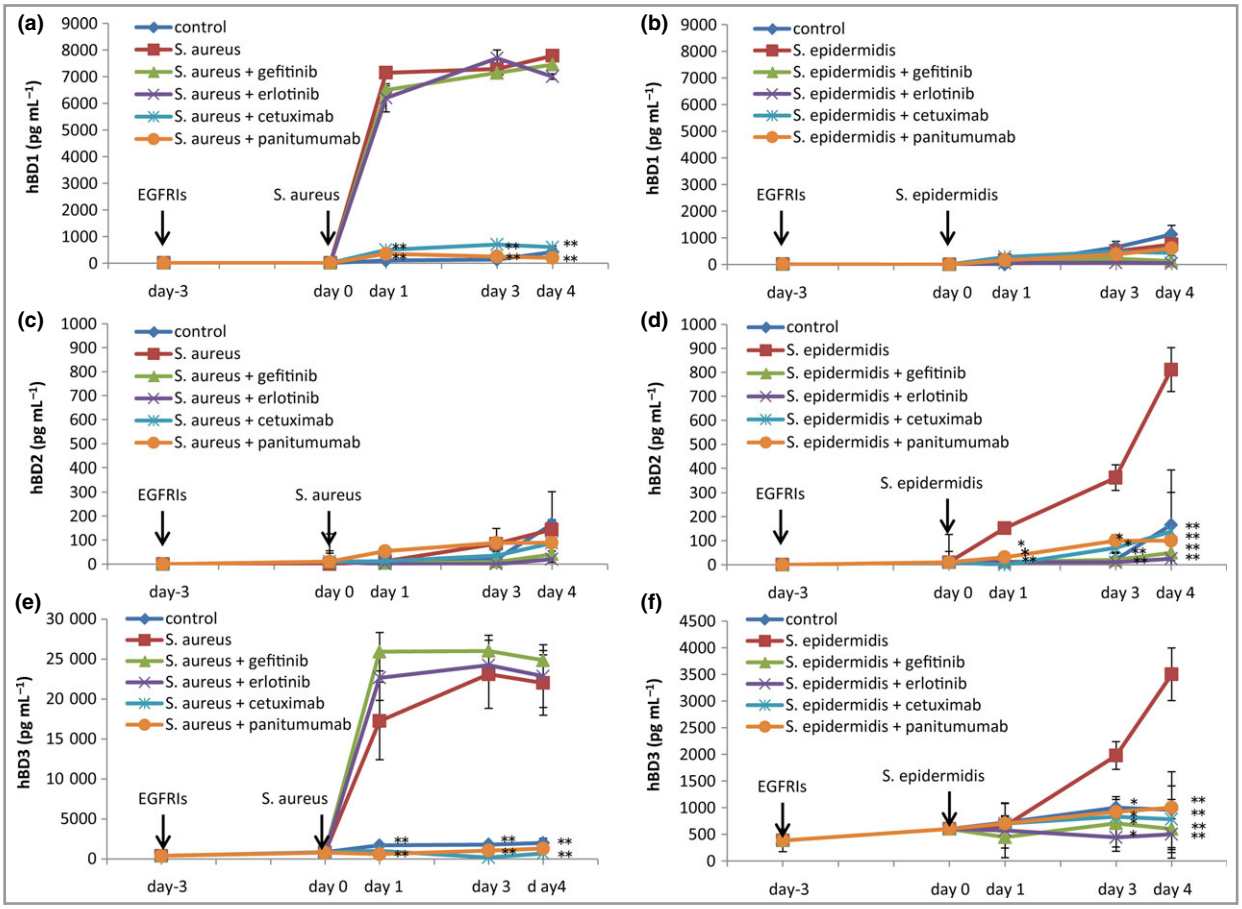

Fig 1. Suppressive effects of epidermal growth factor receptor inhibitors (EGFRIs) on human β-defensin (hBD) expression induced by staphylococci. Normal human epidermal keratinocytes were cultured with or without EGFRIs for 3 days before stimulation with the bacterial supernatants, and the keratinocytes were cultured for an additional 4 days. The expression levels of (a, b) hBD1, (c, d) hBD2 and (e, f) hBD3 in the culture supernatants were evaluated by enzyme-linked immunosorbent assay. Data represent the mean ± SD from three experiments. P-values (bacteria-stimulated keratinocytes cultured with vs. without EGFRIs) were evaluated using Student's t-test. *$P < 0{\cdot}05$; **$P < 0{\cdot}01$.

EGFRIs impaired the expression of antimicrobial peptides.[2,3] It is known that papulopustular eruptions caused by EGFR mAbs are more severe (5·2% at least grade 3) than those caused by EGFR TKIs (1·6% at least grade 3).[4,5] In addition, *S. aureus* is frequently detected in papulopustular eruptions caused by EGFRIs.[4] This led us to hypothesize that EGFR mAbs block hBD expression by keratinocytes in a way different from EGFR TKIs. In this study, we demonstrated that this is actually the case; EGFR mAbs blocked the expression of all hBDs induced by both *S. aureus* and *S. epidermidis*.

Normal human epidermal keratinocytes (NHEKs) were cultured in the presence or absence of the EGFRIs for 3 days before secreted products of *S. aureus* and *S. epidermidis* were added into the culture. We used the EGFR TKIs gefitinib (1·25 μg mL^{-1}) or erlotinib (5 μg mL^{-1}), and the EGFR mAbs cetuximab (0·2 mg mL^{-1}) or panitumumab (0·16 mg mL^{-1}). The final concentrations of these drugs were adjusted to the maximum concentrations of each drug in the human blood. The filtrated bacterial culture supernatants, prepared as described previously,[6] were used as the source of the secreted products of staphylococci. The NHEKs stimulated with the secreted products of staphylococci were cultured with or without EGFRIs for an additional 4 days. The expression levels of hBDs in the culture supernatants were then evaluated by enzyme-linked immunosorbent assay, as described previously.[6]

Consistently with the findings of our previous study, the secreted products of *S. aureus* and *S. epidermidis* induced the expression of hBD1 and hBD3 (Fig. 1a, c, e) and hBD2 and hBD3 (Fig. 1b, d, f), respectively.[6] We next examined the effects of EGFR mAbs on the expression of hBD. We found that both EGFR mAbs, cetuximab and panitumumab, suppressed the expression of hBD1 and hBD3 induced by the secreted products of *S. aureus* (Fig. 1a, e), in addition to the expression of hBD2 and hBD3 induced by the secreted products of *S. epidermidis* (Fig. 1d, f). This is in sharp contrast to the effects of EGFR TKIs, which suppressed the expression of hBD2 and hBD3 induced by the secreted products of *S. epidermidis* (Fig. 1d, f), but not expression of hBD1 and hBD3 induced by the secreted products of *S. aureus* (Fig. 1a, e), as previously reported.[1]

Keratinocytes serve as the front line of defence against the invasion of pathogenic microbes, presumably by exhibiting different responses depending on the types of microbes, thereby acting as a crucial site for innate immune response. hBDs are secreted from epithelial cells, including keratinocytes, and function as immunoreactive agents when stimulated by microorganisms.[7] We previously reported that *S. aureus* and *S. epidermidis* induced the expression of distinct subtypes of hBD by keratinocytes.[6] In this study, we demonstrate that hBD production by keratinocytes is differentially regulated by EGFR mAbs and EGFR TKIs when stimulated with staphylococci. In the presence of EGFR mAbs, keratinocytes did not respond to the secreted products of *S. epidermidis* or *S. aureus*, whereas in the presence of EGFR TKIs, keratinocytes demonstrated a significant response to the secreted products of *S. aureus* by producing a certain hBD.

Currently, the mechanism of differential effects between EGFR TKIs and EGFR mAbs is unknown, although their signalling pathways are well characterized.[8] There may be an additional signalling pathway other than the known tyrosine kinase-dependent pathway. Our results suggest that EGFR mAbs and EGFR TKIs have distinct effects on the keratinocyte innate immune response. Marked suppression of *S. aureus*-induced hBDs by EGFR mAbs, but not EGFR TKIs, may cause more severe papulopustular eruptions induced by *S. aureus*. The precise mechanisms of cutaneous adverse effects caused by each EGFRI are still unknown, although they are thought to be related to EGFR blockade in the skin. However, these differential effects of each EGFRI on hBD production may be associated with the severity of the cutaneous adverse reaction. Further study is needed to verify whether the effects of EGFRIs on the innate immune response induced by commensal bacteria are involved in cutaneous adverse reactions in patients.

[1]*Department of Dermatology and* [3]*Oncology Center, Nara Medical University, 840 Shijocho, Kashihara, Nara 634-8522, Japan*
[2]*Department of Dermatology, Yamato Takada Municipal Hospital, Nara, Japan*
Correspondence: Hideo Asada.
E-mail: asadah@naramed-u.ac.jp

R. Ommori[1]
K. Park[2]
F. Miyagawa[1]
H. Azukizawa[1]
M. Kanno[3]
H. Asada[1]

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原著論文　症例報告

急性期に川崎病との鑑別を要した Stevens-Johnson 症候群の 1 例

松本佳保里[1)2)]，福岡　秀記[1)]，富田　晃正[3)]，峠岡　理沙[4)]
稲富　　勉[1)]，上田真由美[1)]，外園　千恵[1)]
[1)]京都府立医科大学眼科学教室，[2)]バプテスト眼科クリニック，[3)]京都府立医科大学小児科学教室
[4)]京都府立医科大学皮膚科学教室

要　約

背　景：Stevens-Johnson 症候群（SJS）は急性期に眼病変を生じ，慢性期に重篤な視力障害をもたらすことがある．発症年齢が低いほど急性期の眼所見は重篤化しやすく，発症早期の適切な診断が重要である．当初，小児科で川崎病と考えられていたが皮膚科および眼科受診で SJS と診断された 1 例を経験したので報告する．

症　例：8 歳男児．発熱，咽頭痛，両眼結膜充血を生じたため A 医院を受診し，感冒が疑われ，抗ヒスタミン薬，去痰薬，気管支拡張薬，抗プラスミン薬，第三世代セフェム系抗菌薬を処方された．翌日から咽頭痛が悪化，さらに全身に発疹が出現した．このため B 総合病院を受診したところ川崎病の疑いで入院となった．入院後の眼科検査で偽膜形成を認め，SJS 疑いで当院に転院となった．全身性に融合傾向がある非典型的ターゲット状紅斑，血痂と出血を伴う口腔口唇粘膜の発赤腫脹とびらん，両眼の偽膜形成，結膜上皮欠損から SJS と診断された．ステロイドパルスおよびベタメタゾン点眼などで治療を行ったところ，奏効し，視力障害を残さず治癒した．

結　論：SJS の早期診断，治療には皮膚科および眼科の診察が大切である．（日眼会誌 122：705–710，2018）

キーワード：Stevens-Johnson 症候群（SJS），中毒性表皮壊死症（TEN），川崎病，小児，上皮欠損

Original Article　Case Report

A Case of Stevens-Johnson Syndrome Requiring Differentiation from Kawasaki Disease in the Acute Phase

Kaori Matsumoto[1)2)], Hideki Fukuoka[1)], Akimasa Tomida[3)], Risa Mineoka[4)]
Tsutomu Inatomi[1)], Mayumi Ueta[1)] and Chie Sotozono[1)]
[1)]*Department of Ophthalmology, Kyoto Prefectural University of Medicine*
[2)]*Baptist Eye Clinic*
[3)]*Department of Pediatrics, Kyoto Prefectural University of Medicine*
[4)]*Department of Dermatology, Kyoto Prefectural University of Medicine*

Abstract

Background：**Stevens-Johnson syndrome (SJS) causes ocular disease in the acute stage and severe visual impairment in the late stage. Severe ocular findings during the acute phase are more likely to be noted when the age of onset is younger, making early, appropriate diagnosis important. Herein, we report the case of a patient who was initially diagnosed with Kawasaki disease by a pediatrician and was later correctly diagnosed with SJS by a dermatologist and an ophthalmologist.**

Case report：**The patient was an 8-year-old boy who initially presented at Clinic A due to a fever, sore throat, and conjunctival hyperemia in both eyes. Antihistamine agents, expectorants, bronchodilators, antiplasmin agents, and third-generation cephem antibiotics were administered because of presumed common cold-like symptoms. However, his sore throat worsened the following day, and a systemic rash developed over his entire body. He subsequently visited General Hospital B and was admitted with**

連絡責任者：606–8287　京都市左京区北白川上池田町 12　バプテスト眼科クリニック　松本佳保里
（平成 29 年 12 月 25 日受付，平成 30 年 3 月 26 日改訂受理）
Corresponding author：　Kaori Matsumoto, M. D.　Baptist Eye Clinic. 12 Kamiikeda-cho, Kitashirakawa, Sakyo-ku, Kyoto 606–8287, Japan
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suspected Kawasaki disease. After admission, the on-duty ophthalmologist discovered that he was afflicted with bilateral pseudomembranous conjunctivitis, and he was then transferred to our hospital due to suspected SJS. He was finally diagnosed with SJS due to atypical target-shaped erythema, flare/swelling and erosion of oral/labial mucosa with blood crust and bleed, bilateral pseudomembranous conjunctivitis, and conjunctival epithelial defect. We successfully treated the patient with pulse steroid therapy and the topical administration of betamethasone eye drops, with no continued visual impairment.

***Conclusion*：Examination by both a dermatologist and an ophthalmologist is useful for the early diagnosis and treatment of SJS.**

Nippon Ganka Gakkai Zasshi (J Jpn Ophthalmol Soc) 122：705–710, 2018.

Key words：Stevens-Johnson syndrome (SJS), Toxic epidermal necrolysis (TEN), Kawasaki disease, Pediatrics, Epithelial defect

Ⅰ 緒 言

Stevens-Johnson 症候群(Stevens-Johnson syndrome：SJS)は 1922 年にアメリカの小児科医である A. M. Stevens と F. C. Johnson によって，出血性麻疹と診断されていた 2 症例が新たな疾患として報告された[1]．その後，皮膚科の大規模調査から SJS と中毒性表皮壊死症(toxic epidermal necrolysis：TEN)が同じスペクトルの疾患であることが明らかになった[2)~4)]．

SJS は，高熱や全身倦怠感などの症状を伴って，口唇・口腔，眼，外陰部などを含む全身に紅斑・びらん・水疱が多発し，表皮の壊死性障害を生ずる疾患である[5)6)]．原因は多くが薬剤性とされるが，先行するマイコプラズマ感染[7)8)]やヘルペス，麻疹などのウイルス感染が誘因である可能性も指摘されている[9)]．高熱，全身倦怠感，食欲低下などの全身症状に加え，全身の滲出性紅斑や水疱形成を伴う紅斑などの皮膚症状が認められる．眼球結膜の充血と偽膜形成，角結膜上皮欠損が典型的な眼科的急性期所見であり，SJS/TEN の国内 135 例の調査では，これらの重篤な眼合併症が急性期患者の約 40% に認められた[10)]．また，発症年齢が低いほど重篤化しやすい傾向であった[10)]．発症後に副腎皮質ステロイド(以下，ステロイド)点眼を開始した群はしなかった群よりも視力予後が良好であったことから[11)]，できるだけ早期に診断して治療を開始することが重要と考えられる．

川崎病は 1967 年に小児科医川崎富作らにより初めて報告された[12)]．それまでは SJS を含む眼皮膚粘膜症候群として治療されていた症例の中に川崎らの報告した疾患が含まれると考えられた[12)]．その後，厚生省研究班が設置され，川崎病診断の手引きが作成された．それをもとに実施された全国調査の結果，予後良好と考えられていた川崎病に突然死する例の存在が明らかになった．さらにその死亡例が，従来からアメリカで報告されていた乳児結節性動脈周囲炎死亡例の病理解剖と一致したことから，臨床像からみた眼皮膚粘膜症候群と病理像からみた乳児結節性動脈周囲炎を合わせたものが川崎病の概念となった．先行する感染症や遺伝子が原因とする報告も散見されるが，川崎病の発症原因は未だに不明である．川崎病の罹患患者数は，近年の少子化にもかかわらず，15,000 人/年と増加傾向にある．全身の血管炎が基本病態であり，発熱，発疹，口唇・口腔の発赤，リンパ節腫脹などを認める疾患である．合併症として両眼性に球結膜充血や虹彩毛様体炎などの眼病変や突然死のリスクがある心障害を認め，発症 1 か月以内の急性期心障害は全体の 8.5% で認められた[13)]．

今回，当初小児科で川崎病と考えられていたが，皮膚科および眼科診察により SJS に特徴的である非典型的ターゲット状紅斑，血痂と出血を伴う口唇びらん，両眼結膜充血，偽膜形成，結膜上皮欠損を認めたため SJS と診断，治療され，重篤な後遺症を残さず治癒した症例を経験したので報告する．本報告はヘルシンキ宣言を遵守し，京都府立医科大学医学倫理審査委員会の承認を受けて行った．

Ⅱ 症 例

8 歳男児，生来健康であり既往歴はなかった．12 月 23 日から 37.5℃の発熱と咽頭痛，軽度両眼結膜充血を認めた．翌日に近医 A 医院を受診し，抗ヒスタミン薬，去痰薬，気管支拡張薬，抗プラスミン薬，第三世代セフェム系抗菌薬を処方され，同日より内服を開始した．翌日には新たに手足の発疹と咽頭痛の悪化を認め，翌々日には 39℃の発熱，発疹と両眼結膜充血の悪化，新たな口腔内びらんを生じたため，B 総合病院小児科を受診した．川崎病診断基準(表 1)の主要症状 6 項目のうち 4 項目(発熱，両側眼球結膜，口唇・口腔所見，不定形発疹)が合致するため，川崎病の疑いとして精査加療目的に B 総合病院に入院した．入院時に四肢の浮腫や紅斑，頸部リンパ節腫脹を認めたが，感染性疾患が原因となる化膿性頸部リンパ節腫脹である可能性も否定できず，川崎病の主要症状に該当するものであるかどうかは小児科医の間で見解が分かれた．入院後に両眼結膜充血のさらなる悪化と眼脂が出現したため，入院翌日に B 総合病院眼科を受診した．川崎病には合致しないと考えられる両眼瞼結膜の偽膜形成を認めたため，川崎病ではなく SJS の疑いで精

表 1　川崎病の診断基準

主要症状
1．5 日以上続く発熱
2．両側眼球結膜の充血
3．口唇，口腔所見：口唇の紅潮，いちご舌，口腔咽頭粘膜のびまん性発赤
4．不定形発疹
5．四肢末端の変化：
　(急性期)手足の硬性浮腫，掌蹠ないしは指趾先端の紅斑
　(回復期)指先からの膜様落屑
6．急性期における非化膿性頸部リンパ節腫脹

・6 つの主要症状のうち 5 つ以上の症状を伴うもの．
・上記 6 主要症状のうち 4 つの症状しか認められなくても，冠動脈瘤(いわゆる拡大を含む)が確認され，他の疾患が除外されれば本症とする．

(2002 年　厚生労働省研究班)

表 2　Stevens-Johnson 症候群(SJS)の診断基準

主要症状
1．皮膚粘膜移行部(眼，口唇，外陰部など)の広範囲で重篤な粘膜病変(出血・血痂を伴うびらん等)がみられる．
2．皮膚の汎発性の紅斑に伴って表皮の壊死性障害に基づくびらん・水疱を認め，軽快後には痂皮，膜様落屑がみられる．
　その面積は体表面積の 10% 未満である．但し，外力を加えると表皮が容易に剝離すると思われる部位はこの面積に含まれる．
3．発熱がある．
4．病理組織学的に表皮の壊死性変化を認める．
5．多形紅斑重症型(erythema multiforme［EM］major)を除外できる．

・副所見を十分考慮の上，主要所見 5 項目をすべて満たす場合を SJS と診断する．
・初期のみの評価ではなく全経過の評価により診断する．

(日皮会誌 126：1637–1685，2016．)

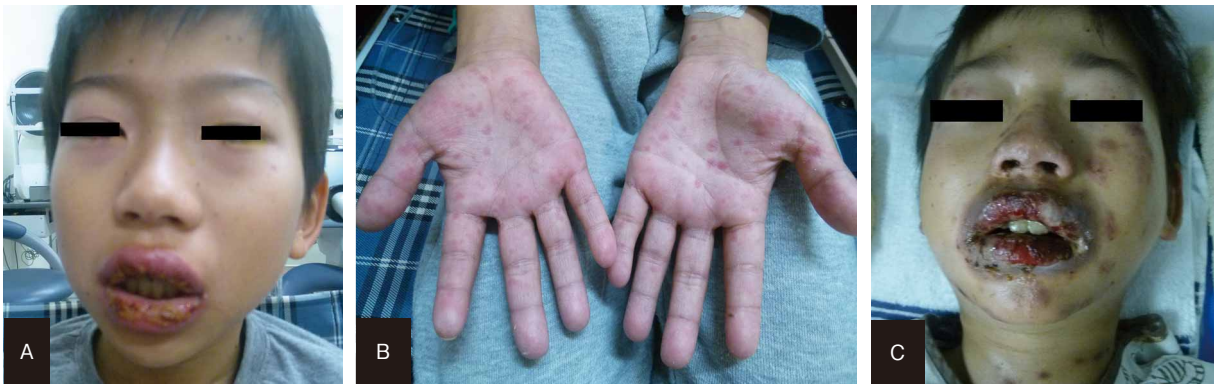

図 1　全身所見．
A：発症後 2 日．口唇は発赤腫脹しびらんを生じている．頬部や眼周囲に発赤を生じている．
B：発症後 2 日．両手掌にはびまん性に SJS に特徴的な発疹を生じている．
C：発症後 6 日．口唇の発赤腫脹は増悪し一部で痂皮化がみられる．

査加療目的に京都府立医科大学附属病院に紹介となった．

初診時所見：SJS 診断基準(表 2)の主要項目 5 項目のうち 3 項目(粘膜病変，表皮のびらん・水疱，発熱)を満たした．38℃を超える発熱，全身性に融合傾向がある非典型的ターゲット状紅斑，血痂と出血を伴う口腔口唇粘膜の発赤腫脹とびらん(図 1)，両眼に結膜充血と眼瞼の偽膜形成，球結膜上皮欠損を認めた(図 2)．アデノウイルス結膜炎や沐様状結膜炎などの偽膜を形成する他疾患を鑑別する必要があるが，結膜びらんや眼瞼縁のびらんを同時に生じる疾患は SJS 以外にない．また，今回の症例では，SJS に特徴的な所見である爪周囲の炎症所見がみられた．血液検査像では，免疫グロブリンが IgG 739 mg/dL とやや低値で，C-reactive protein (CRP) 6.11 mg/dL，アルブミン 4.0 g/dL，prothrombin time-international normalized ratio(PT-INR) 1.20，尿中白血球(2+)であり，これらは川崎病の診断における参考条件と合致した．眼科医，皮膚科医，小児科医の診察で，皮膚所見と副所見である眼病変から SJS と診断し速やかにステロイドパルス療法とステロイド局所治療を開始する方針となった．

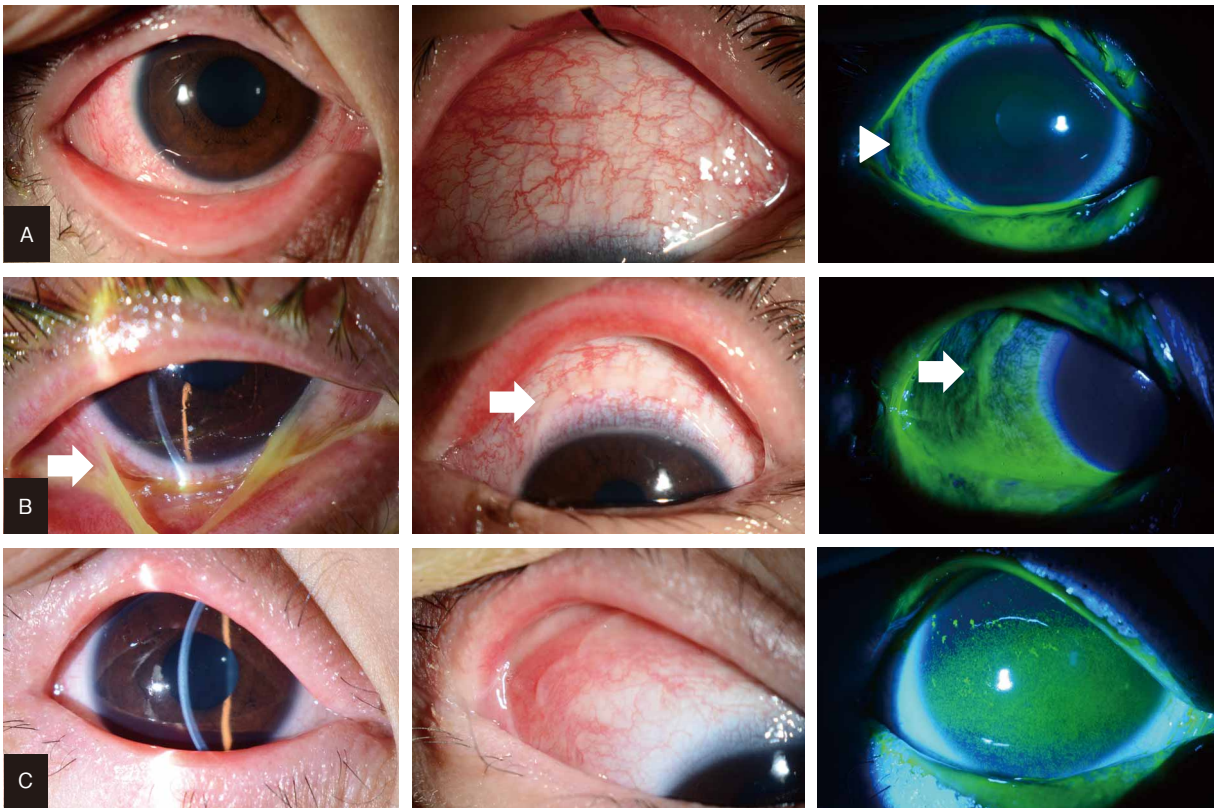

図 2 眼所見の経過.
A：発症後2日(当院初診時)．結膜充血，偽膜形成，結膜上皮欠損(白矢頭)がみられる．
B：発症後7日．偽膜形成(白矢印)がみられ偽膜除去を行った．
C：発症後1か月．結膜充血は改善し偽膜は消失した．フルオレセイン染色で点状表層角膜症を認める．

入院後経過：当院入院日(発症後2日)からステロイド全身および局所投与を開始した．まず入院日にはメチルプレドニゾロン300 mg/日を点滴投与し，翌日からはメチルプレドニゾロン600 mg/日を3日間点滴投与した．ステロイドパルス療法終了後はプレドニゾロン50 mg/日内服に漸減したが，全身の発疹やびらんは拡大傾向であり免疫グロブリン療法を引き続き1クール(5日)併用した．発症後2週で全身の水疱は徐々に痂皮化しはじめた．全身の皮膚粘膜病変の病勢に合わせてステロイドを漸減し，ステロイドの全身投与は約1か月で終了とした(図3)．

眼病変に対しては，当院入院時から0.1% ベタメタゾンリン酸エステルナトリウム点眼8回/日とベタメタゾンリン酸エステルナトリウム・フラジオマイシン硫塩酸(リンデロン®A)眼軟膏4回/日のステロイド局所投与と，ガチフロキサシン点眼4回/日の局所療法を開始し，発症後30日まで継続した．両眼眼球結膜充血と偽膜形成，結膜上皮欠損は入院後に増悪，発症後1週でピークを迎え，徐々に改善した．発症後1か月にも両眼結膜充血と偽膜形成は残存していたが，その後は発症後41日で退院となり，発症後57日には完全に消失した．ステロイド治療が原因と考えられる眼圧上昇が発症後1か月でみられたため，2% カルテオロール塩酸塩点眼を開始し，リンデロン®A眼軟膏を漸減，0.1% ベタメタゾンリン酸エステルナトリウム点眼を0.1% フルオロメトロン点眼に変更した．ステロイド投与量の漸減に伴い，眼圧も正常化した．なお，2005年7月から2016年12月までの間に当院で発症初期にステロイドパルス療法とベタメタゾンリン酸エステルナトリウム局所投与を行った10例20眼中3例3眼で一過性の眼圧上昇がみられたものの，本症例と同様にステロイド漸減に伴い速やかに眼圧は正常化し，緑内障に至った症例はなかった．

発症後2日に施行した皮膚生検では表皮の全層性に壊死性変化がみられ，リンパ球・形質細胞・好中球の浸潤が認められSJSに矛盾しない病理所見が得られた(図4)．そして，発症後16日に施行した薬剤リンパ球刺激試験では検査を行ったセフジニル細粒とカルボシステインとトランサミンシロップのすべてで陽性となったが，皮疹消退後の発症後34日に施行した同検査ではすべてで陰性となった．

発症後3か月経過時点では，Schirmer試験が右眼2 mm，左眼22 mmと涙液減少がみられ，フルオレセイン染色で点状表層角膜症がみられた．矯正視力は右(1.0×−1.75 D⊂cyl−1.00 D Ax 15°)，左(1.0×−1.75 D)と良好で，全身的な後遺症を残さなかった．

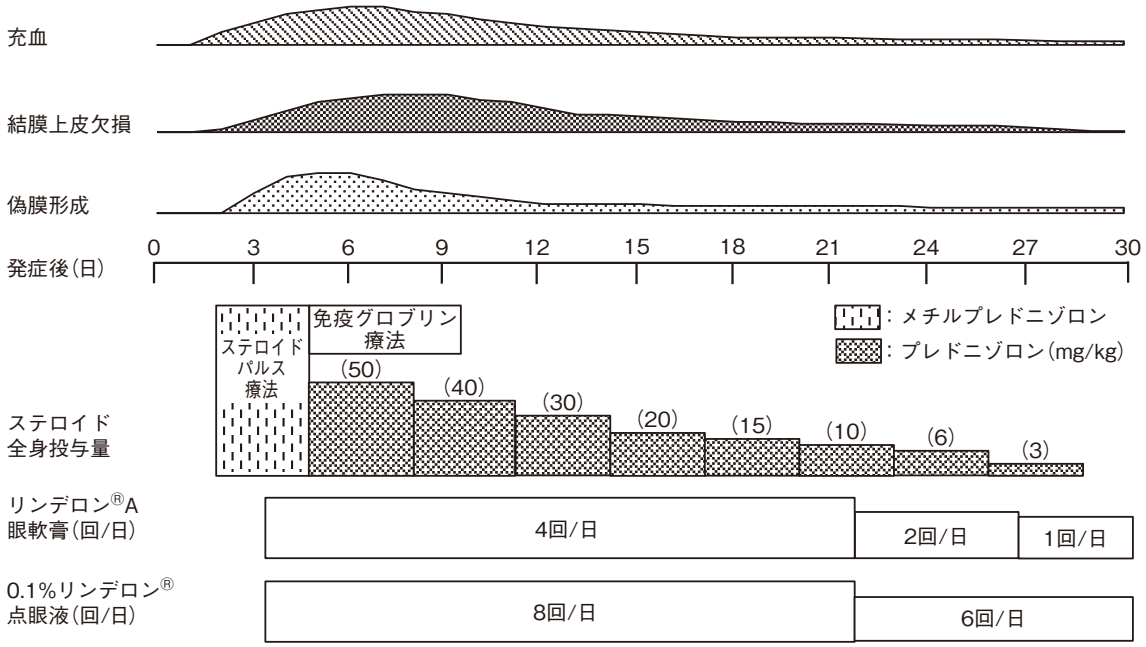

図 3　治療経過.

発症後 2 日からステロイドパルス療法を開始した．同時にベタメタゾンリン酸エステルナトリウム(リンデロン®)点眼と眼軟膏による局所治療も開始した．ステロイドパルス療法後も粘膜病変の増悪がみられたため免疫グロブリン療法を併用した．その後は全身状態と眼所見の改善とともにステロイド投与量を漸減した．

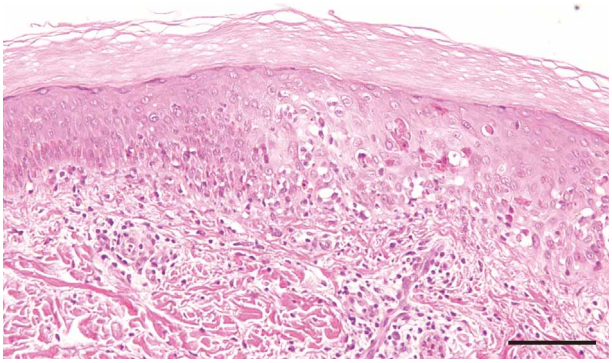

図 4　皮膚生検の病理組織所見.

下腿紅斑の生検で病変部には真皮表皮接合部に空胞形成と炎症細胞の浸潤を認め，表皮の壊死像が明らかである．スケールバーは 100 μm.

Ⅲ　考　　按

厚生労働省の重症多形滲出性紅斑に関する調査研究班(難治性疾患克服研究事業)により「SJS および TEN の治療指針 2007」が作成され，2016 年には，皮膚および粘膜病変への局所療法について新たに治療指針が追記された[6]．SJS の標準治療は，ステロイドパルス療法とベタメタゾンリン酸エステルナトリウム投与での治療である．眼表面上皮欠損や偽膜形成を認める際は，慢性期に視力障害などの重篤な後遺症が残るリスクが高く[10]，消炎のためにベタメタゾンリン酸エステルナトリウムの局所療法が推奨される．

今回，川崎病として治療されていた症例が眼所見から SJS と判明し治療を開始したところ良好な結果を得た．そこで川崎病と SJS の違いについて比較を行った．SJS の発症頻度は人口 100 万人当たり年間 3.1 人[5]，川崎病の罹患患者は全国で年間約 15,000 人[13]と報告されており，15 歳未満の小児に限って発症頻度を比較すると，川崎病は SJS の約 300 倍起こりやすい．また川崎病が小児で発症するのに対し，SJS は全年齢で発症する特徴がある．小児科医においては SJS よりも川崎病のほうが診療の機会が格段に多いといえる．また SJS の病態は，表皮や粘膜上皮の壊死性障害を特徴とするのに対し，川崎病の病態は全身性の血管炎を特徴とし，全身の発疹や眼球結膜充血を認めるなど一見すると非常に臨床症状は類似しているため鑑別が難しいことがあるのも理解できる．

SJS 治療の第一選択は高用量ステロイド全身投与および局所療法である．一方，川崎病治療の第一選択は免疫グロブリン大量療法と抗血小板薬である．ステロイド薬の単剤投与により冠動脈病変の発症率が上昇する可能性があるため冠動脈病変を伴う川崎病へのステロイド投与は禁忌であると考えられており[14]，SJS と川崎病では治療方針が異なる．現在では川崎病に対する免疫グロブリン療法不応例ではステロイドパルス療法で急性期の病勢を沈静化させる治療が見直されつつあるが，本症例では川崎病としてアスピリン投与による初期治療が行われていた．我々のグループは，SJS の発症早期にステロイドパルス療法を行うことで重篤な後遺症を予防できると報告した[15]．本症例においても，速やかに発症 2 日でメチ

ルプレドニゾロン300 mg/日のミニパルス療法を，発症3日からはメチルプレドニゾロン600 mg/日のパルス療法を3日間施行した．同時に局所ステロイド投与を行い上皮欠損と偽膜形成は極期を迎えた．発症早期にステロイドパルス全身投与とステロイド局所治療を施行していたことで，徐々に病勢は落ち着き，重篤な後遺症を残すことなく治癒した．本症例ではステロイドパルス療法後もすぐには病勢が衰えず，免疫グロブリン療法を1クールのみ併用した．川崎病と診断され免疫グロブリン療法のみで治療を行った場合も軽快して治癒した可能性はあるが，眼科医が診療に関わらなかった場合，重篤な眼後遺症が残った可能性がある．

SJSの発症初期は発疹のみを呈する場合があり，小児例では川崎病との鑑別が困難な場合がある．SJSは粘膜の壊死性障害としての偽膜性結膜炎や上皮欠損を認めるが，川崎病は両眼の眼球結膜充血のみで偽膜性結膜炎や上皮欠損を認めないことが大きな相違であり鑑別に有用である．SJSは，重篤な眼後遺症を防ぐための発症早期での全身療法と局所療法が重要である．皮膚症状については，SJSでは浮腫が少なくて三層構造が明瞭でないターゲット様紅斑がみられる．一方で，川崎病では主要症状である不定形発疹がみられるが，その発疹は風疹・麻疹様の丘疹・紅斑や蕁麻疹様の膨疹などさまざまで，多形紅斑の臨床像を呈することもあり[16]，SJSと鑑別が難しい場合がある．口唇部については，川崎病では口唇部の発赤，腫脹，乾燥を認めるが，SJSでは口腔内から口唇にかけて血痂や出血を伴うびらんを認めることが多く，川崎病より強い粘膜障害を認める傾向がある．川崎病ではみられないSJSに特異的な所見を早期に発見するには，刻々と変化する病勢の適切な時期に眼科医および皮膚科医による診察が必要と考えられる．

利益相反：外園千恵(カテゴリーF：参天製薬，カテゴリーP)

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原著論文 症例報告

白内障手術後の前嚢縁白色塊から術後真菌性眼内炎の診断に至ったStevens-Johnson症候群の1例

吉岡 誇[1)2)]，福岡 秀記[2)]，米田 一仁[2)]，外園 千恵[2)]

[1)]市立福知山市民病院眼科，[2)]京都府立医科大学眼科学教室

要 約

目 的：水晶体再建術後に真菌性眼内炎を来し，治療に苦慮したStevens-Johnson症候群(SJS)の症例を経験したので報告する．

症 例：59歳，SJSの女性であり，加齢白内障が進行したため水晶体再建術を施行した．術前の結膜嚢擦過培養検査でカンジダ属を検出したため周術期に0.1%ミコナゾール硝酸塩(MCZ)点眼を使用した．経過良好にて消炎を得たため，術後34日にMCZ点眼を終了したところ，前房内炎症が再燃し，散瞳検査により前嚢縁に白色塊を認めた．術後91日に前嚢切除術を施行し，前嚢よりカンジダ属を検出したため術後真菌性眼内炎と診断した．MCZ点眼およびボリコナゾール内服による治療を開始し，改善を得たが副作用と考えられる視力低下を認め内服を中止した．それに伴い眼内炎が増悪し，白内障手術後7か月において硝子体手術および眼内レンズ摘出術を施行した．術後に0.1% MCZ点眼およびイトラコナゾール内服を行い，治癒した．

結 論：難治性眼表面疾患に対する内眼手術時には，日和見感染や耐性菌による眼内炎の予防に注意を要する．(日眼会誌 122：928–933，2018)

キーワード：水晶体再建術，真菌感染症，眼内炎，カンジダ属，Stevens-Johnson症候群

Original Article Case Report

Postoperative Fungal Endophthalmitis Diagnosed Using Culture of a White Mass on the Edge of the Anterior Capsule Following Cataract Surgery in a Stevens-Johnson Syndrome Patient：a Case Report

Hokoru Yoshioka[1)2)], Hideki Fukuoka[2)], Kazuhito Yoneda[2)] and Chie Sotozono[2)]

[1)]*Department of Ophthalmology, Fukuchiyama City Hospital*

[2)]*Department of Ophthalmology, Kyoto Prefectural University of Medicine*

Abstract

***Purpose*：To report a case of refractory Stevens-Johnson syndrome (SJS) in which fungal endophthalmitis developed after lens reconstruction.**

***Case presentation*：The patient was a 59-year-old woman with SJS who underwent lens reconstruction because of the progression of an age-related cataract. As preoperative culture of conjunctival sac swab revealed the presence of *Candida* spp., eye drops containing 0.1% miconazole (hereafter MCZ) were perioperatively administered. As the patient progressed well and the ocular inflammation subsided, the MCZ eye drops were discontinued on postoperative day 34. However, intracameral inflammation reappeared and a white mass was identified in the anterior capsule edge according to testing performed with dilated pupils. Anterior capsulectomy was performed on postoperative day 91 and a diagnosis of postoperative fungal endophthalmitis was made based on the detection of *Candida* spp. in the anterior capsule. Treatment was initiated with 0.1% MCZ eye drops and oral voriconazole and improvement was noted. However, as decreased visual acuity that appeared to be a side effect of voriconazole appeared, the drug was discontinued. As the intraocular inflammation subsequently worsened, vitrectomy and intraocular lens extirpation were performed seven months after the cataract surgery. Postoperatively, the fungal endophthalmitis was successively treated by administering 0.1% MCZ eye drops and oral itraconazole.**

***Conclusion*：When performing intraocular surgery on patients with refractory ocular surface disease, care must be taken to prevent opportunistic infections and endophthalmitis due to resistant bacteria.**

Nippon Ganka Gakkai Zasshi (J Jpn Ophthalmol Soc) 122：928–933, 2018.

Key words：Lens reconstruction, Fungal infection, Endophthalmitis, *Candida* spp., Stevens-Johnson syndrome

連絡責任者：620–8505 福知山市厚中町231 市立福知山市民病院眼科 吉岡 誇

(平成30年2月13日受付，平成30年6月20日改訂受理) E-mail：hokoru@koto.kpu-m.ac.jp

Corresponding author： Hokoru Yoshioka, M. D. Department of Ophthalmology, Fukuchiyama City Hospital. 231 Atsunaka-cho, Fukuchiyama-shi, Kyoto-fu 620–8505, Japan

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Ⅰ 緒　言

白内障手術後眼内炎の発症率はおよそ 0.025%～0.04% と報告されている[1)~7)]．その中でも，真菌性眼内炎は細菌性眼内炎に比べて頻度は少ないが，一般的に予後不良である．

Stevens-Johnson 症候群(Stevens-Johnson syndrome：SJS)は，突然の発熱と皮膚病変に加えて全身の粘膜にも水疱やびらんを生じる疾患であり，主として薬剤副作用により発症する．後遺症として瘢痕性角結膜上皮症，重症ドライアイや非特異的な慢性炎症を来すため，慢性期の眼科治療として抗菌薬点眼や低濃度副腎皮質ステロイド点眼を長期に使用する[8)]．

今回，白内障手術後に前嚢縁の真菌塊を形成し，前嚢の培養検査により術後真菌性眼内炎と判明したが治療に苦慮した SJS の 1 例を経験したので報告する．

Ⅱ 症　例

患者：59 歳，女性．

既往歴：22 歳時に膀胱炎治療薬の内服後 SJS を発症し，角膜混濁と視力低下を来した．

アレルギー性鼻炎のため近医から処方されたメチルプレドニゾロン 2 mg を長期内服していた．

眼科既往歴：44 歳時に右眼角膜穿孔となり，治療用ソフトコンタクトレンズ装用により治癒した．56 歳時に左眼角膜穿孔となり，結膜被覆術を施行された．視力向上を目的に 2014 年 8 月に京都府立医科大学眼科に紹介された．視力は右(0.15× +2.50 D〇cyl −4.00 D Ax 180°)，左(0.15× −1.00 D)であり，右眼角膜に上皮不整があったためハードコンタクトレンズ(HCL)装用を開始し，右(0.5×HCL＝S＋1.5 D)と視力向上を得た．

経過：前医から処方されていた 0.5% セフメノキシム塩酸塩点眼液，0.02% フルオロメトロン点眼液，防腐剤無添加 0.3% 精製ヒアルロン酸ナトリウム点眼液，人工涙液型点眼剤(ソフトサンティア®)にて管理され，経過良好であった．左眼の白内障が進行し，視力が左 0.2(矯正不能)と低下したため水晶体再建術を予定した．術前の結膜嚢擦過培養検査にて *Candida* spp. を検出したため術前点眼として 0.1% ミコナゾール硝酸塩(MCZ)点眼液(自家調製点眼薬)の投与を行った(以後の副腎皮質ステロイド・抗真菌薬の全身および局所投与による治療経過を図 1 に示す)．

2016 年 10 月に左眼水晶体再建術を強角膜切開で施行し，視認性が不良であったため術中に耳下側の被覆結膜を剝離したが，問題なく終了した．また，術後の眼表面炎症の制御目的で，手術当日からベタメタゾンを点滴・内服で使用した．眼局所には，抗菌薬点眼に加えて，0.1% ベタメタゾンリン酸エステルナトリウム点眼液，0.1% MCZ 点眼液を投与した(図 1)．

術後経過良好であり，消炎を得たため抗菌薬・副腎皮質ステロイド点眼を漸減し術後 34 日に MCZ 点眼を終了したところ，術後 49 日に紹介元の眼科にて軽度前房炎症および角膜後面沈着物を認めたため，虹彩炎を疑われて 0.1% ベタメタゾンリン酸エステルナトリウム点眼液が増量された．術後 62 日の当院受診時に視力は左(0.9× +0.25 D〇cyl −0.75 D Ax 180°)と良好であったが，前房炎症が遷延しており角膜後面沈着物および散瞳検査により前嚢切開部 4～7 時方向の辺縁に白色塊(図 2)の出現を認めた．術後真菌性眼内炎を疑い 0.1% ベタメタゾン点眼液を漸減するとともに 0.1% MCZ リン酸エステルナトリウム点眼液，ボリコナゾール(VRCZ)内服による治療を開始した．

再診時に前房炎症の改善を認めたが，真菌塊である可能性を考慮し，前嚢白色塊の除去および組織診断を目的として前嚢切除術を術後 91 日に施行した．術中に採取した前嚢白色塊の培養検査にて *Candida* spp. を検出した．また，前嚢白色塊の残存を認めたため前嚢切除後 7 日に追加切除した．前嚢切除後 11 日時点での視力は右(0.5×Pin Hole)，左(0.5×Pin Hole)であった．一時，薬剤毒性による上皮欠損を認めたが，抗真菌薬を含め点眼を減量し，1% ピマリシン眼軟膏を追加することで上皮障害は治癒した(図 1)．その後の経過は良好であったが，前嚢切除術後 41 日に右 0.2(矯正不能)，左 0.2(矯正不能)と両眼の視力低下を認めた．VRCZ 内服による副作用を疑い，内服を漸減して前嚢切除術後 111 日に休薬した．このとき，眼内炎症はほとんどなかったが，10 時方向と 6 時方向の前嚢縁にわずかな白色混濁を認めたため注意深く経過観察した(図 3)．感染制御のため，アレルギー疾患に対して以前より長期処方されていたメチルプレドニゾロン内服を耳鼻科主治医との相談のうえで休薬した(図 1)．

前嚢切除後 124 日に眼痛・充血のため当院救急外来を受診した．強い前房内炎症，多量のフィブリン塊，前房蓄膿を認め，感染性眼内炎の増悪を疑い眼内レンズ摘出および網膜硝子体手術を緊急で施行した(図 4A)．

術中に水晶体嚢，眼内レンズおよび硝子体を採取し，塗抹・培養検査および polymerase chain reaction(PCR)を行った．眼内灌流液に VRCZ を添加し，濃度は角膜内皮障害を来さないとされる 10 μg/0.1 mL に調製した[9)]．耳下側の毛様体部の硝子体混濁が認められ，同部位を十分に郭清し，手術を終了した．摘出した眼内レンズおよび残存水晶体嚢の塗抹検鏡により酵母型真菌を多数認め(図 5)，培養検査にて *Candida* spp. を検出し，前房水の PCR 検査においても *Candida* spp. を検出した．

術後，0.1% MCZ 点眼液に加えて，術当日から術後 4 日まで MCZ を点滴静注し，5 日よりイトラコナゾールの内服を行った．経時的に眼内炎症は鎮静化し，術後 9

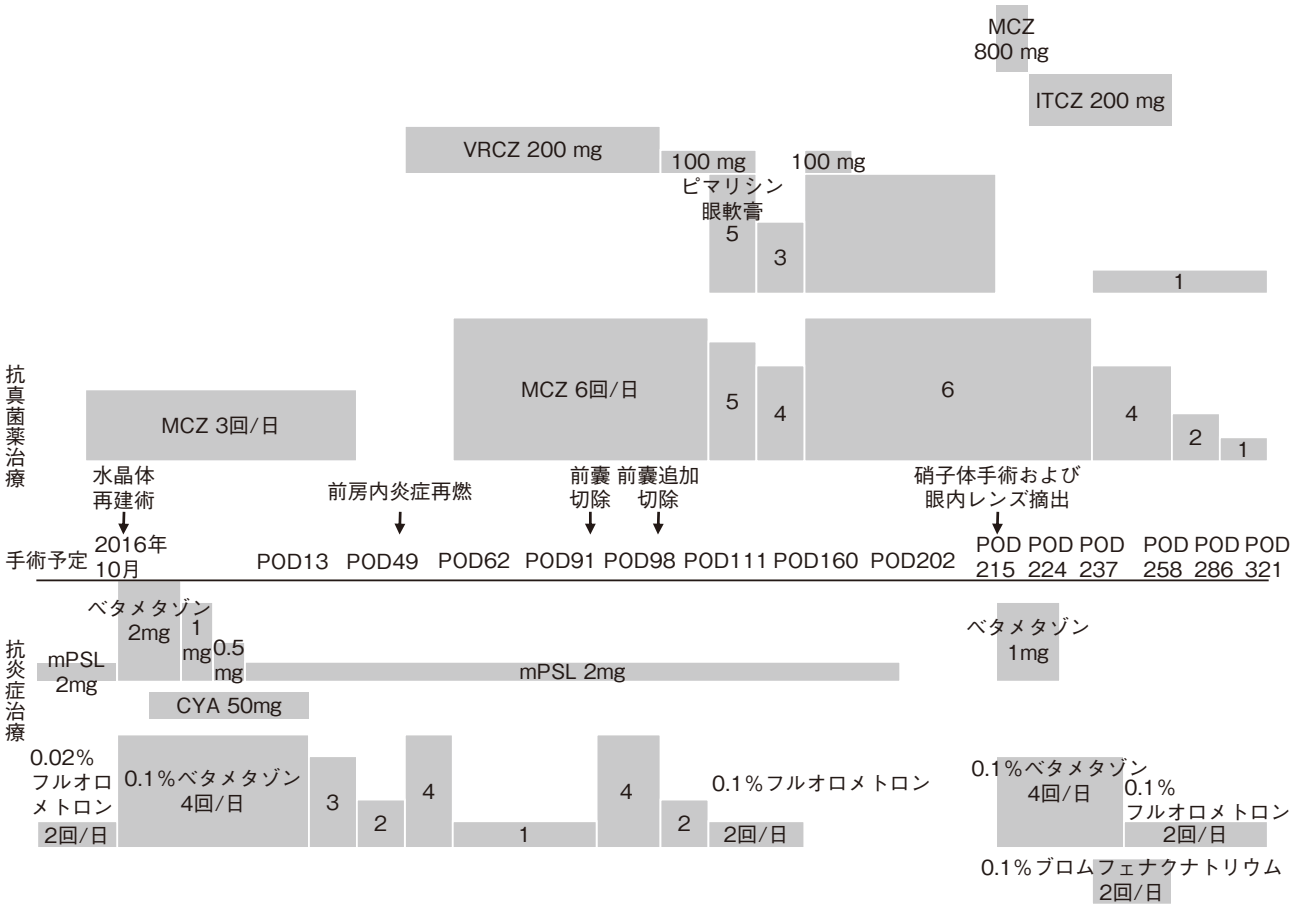

図 1 治療経過.

白内障手術前後に投与した抗真菌薬および副腎皮質ステロイド薬の局所および全身投与経過を表に示す．図内の薬剤は以下のとおり．MCZ：ミコナゾール硝酸塩，VRCZ：ボリコナゾール，ITCZ：イトラコナゾール，mPSL：メチルプレドニゾロン，CYA：シクロスポリン，POD：postoperative day.

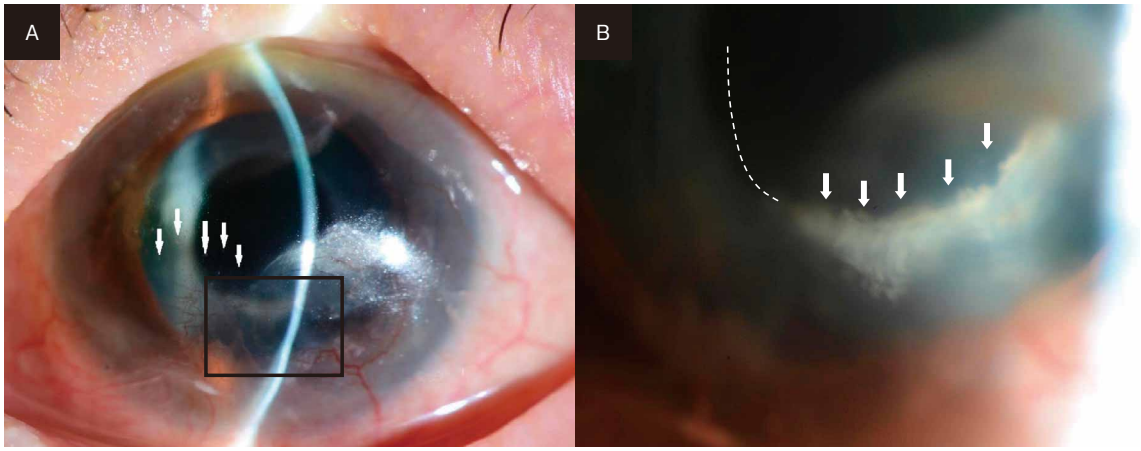

図 2 水晶体再建術後 49 日の左前眼部.

A：充血はないものの，角膜後面沈着物を認める(矢印)．前嚢切開縁に白色塊を認める(黒枠内)．
B：下方の前嚢切開縁(点線)に沿う白色塊を認める(矢印)．

日に採取した前房水 PCR 検査により真菌が検出限界以下であることを確認して，硝子体手術および眼内レンズ摘出術後 11 日に退院した．

その後の経過は良好であり，点眼を漸減し内服を終了した．その後も再燃なく，術後 6 か月における視力は右 0.05(矯正不能)，左 0.08(0.3×＋8.00 D)，HCL 装用時には(0.7×HCL＝S＋14.5 D)と良好であった(図 4B)．

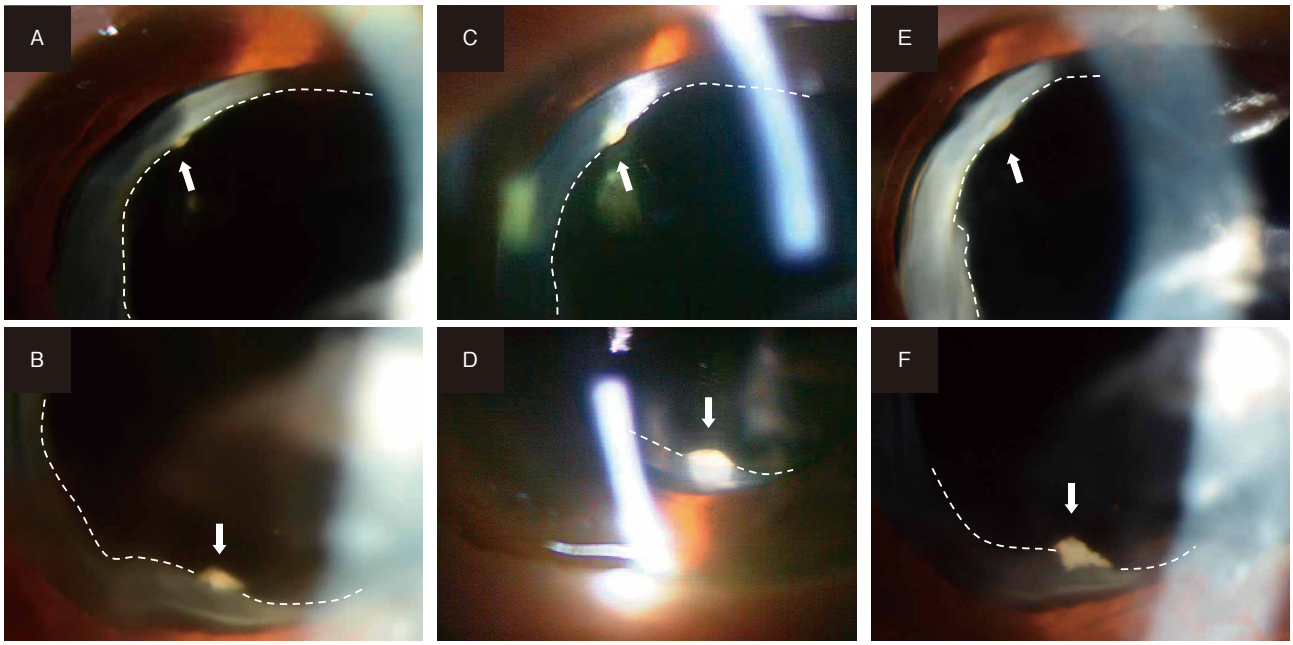

図 3　前嚢切開部の白色塊の経時変化.
左から順に前嚢切除から 69 日(A・B)・83 日(C・D)・111 日(E・F). 前嚢切開縁(点線)の 10 時方向(A・C・E), 6 時方向(B・D・F)の白色塊(矢印)が経時的に増大したが, 眼内炎症はほとんど認めなかった.

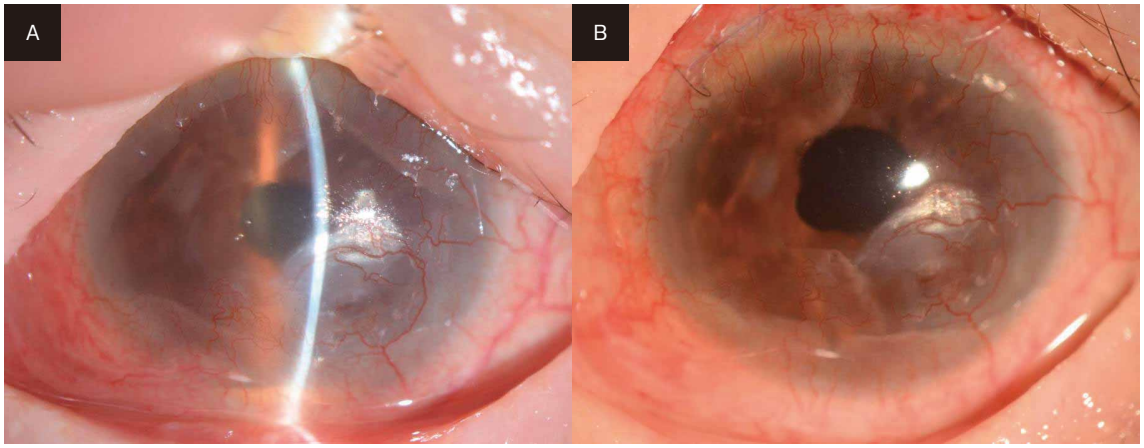

図 4　硝子体手術および眼内レンズ摘出術の前後における左前眼部.
A：前嚢切除術から 124 日に眼痛のため救急受診. 強い前房内炎症, 多量のフィブリン塊, 前房蓄膿を認める.
B：硝子体手術および眼内レンズ摘出術から 6 か月後. 充血や前房内炎症など感染の再発徴候を認めない. 術後, ハードコンタクトレンズ(HCL)装用下で(0.7×HCL＝S＋14.5 D)と視力良好であった.

Ⅲ　考　　按

白内障手術後に真菌性眼内炎を発症した SJS の 1 例を経験した. 術後眼内炎の臨床的特徴として, 真菌性眼内炎は細菌性眼内炎に比べ進行が緩徐であることが多く, 一般的に視力予後は不良である[3)5)10)~12)]. 術後眼内炎と診断されるまでの間に副腎皮質ステロイド点眼による加療によって見かけ上, 炎症の鎮静化が得られ, 感染を遷延させることもある. 今回の症例でも当初は, 炎症増悪に対して副腎皮質ステロイド点眼が紹介元において増量されていた. 今回の症例のように術後晩期に眼内炎症の増悪を認めたときには, 術後眼内炎を疑って, 散瞳検査による精査が必要である.

佐々木らは, 遅発性眼内炎においては, 残存水晶体前嚢と眼内レンズ間に白色プラークを高い率で認めることを報告[11)]している. また, 今回の症例では耳下側の結膜被覆術後の部位に一致して角膜混濁があり, 白内障手術の際に水晶体皮質が残存した可能性を否定できない. 露出したコラーゲン・蛋白質は遊離真菌などが付着しやすく[13)], リスクが高かった可能性がある.

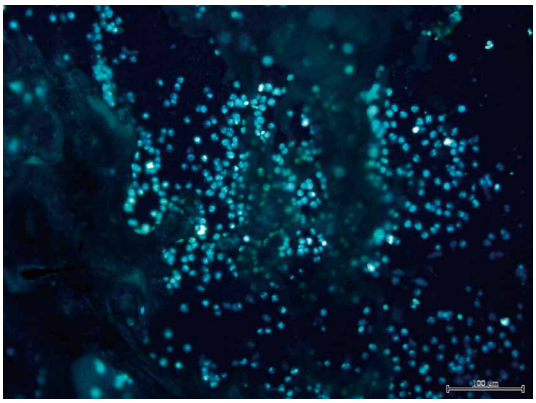

図 5 硝子体手術および眼内レンズ摘出術時に得られた切除検体に対するファンギフローラ Y®染色.
水晶体嚢に付着した多数の酵母様真菌を認める.

本症例では前嚢を白色塊とともに切除することで病巣部を除去できるとともに，真菌感染の診断が可能であった．その後の投薬がある程度有効であったが，完治は困難であった．完治のためには当初より水晶体嚢および眼内レンズ摘出を含めた外科的治療が必要であったと考えられた．白内障手術後の視力が良好であり，眼内炎の自覚症状を認めず，眼内レンズ摘出を決断することが困難であったが，早期の摘出を検討してもよかったかもしれない．

これまでSJS患者における白内障手術後眼内炎についての報告は我々の知るところない．術前の結膜嚢擦過培養検査にて *Candida* spp. を検出し，経過中の前嚢および硝子体からも *Candida* spp. を検出したことから，白内障手術時の眼内への真菌の持ち込みが強く疑われる．術前には長期間にわたりアレルギー性鼻炎に対し副腎皮質ステロイドの内服を処方されており，免疫能低下を来していたことも術後眼内炎を引き起こした要因であったと考えられる[11]．SJSをはじめとした難治性眼表面疾患の患者では，多くは長期にわたり副腎皮質ステロイドや抗菌薬点眼を使用されており，結膜嚢に真菌や耐性菌を保菌している可能性がある．そのため内眼手術に関して，日和見感染や耐性菌による術後眼内炎の予防に注意することが必要である．

利益相反：外園千恵(カテゴリーF：参天製薬，カテゴリーP)

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吉岡　　誇（Hokoru YOSHIOKA）

市立福知山市民病院眼科.

2014 年京都府立医科大学を卒業し，2014 年より国立病院機構京都医療センターにて初期研修を開始，2016 年に修了．2016 年京都府立医科大学眼科学教室に入局，2017 年から市立福知山市民病院眼科に勤務.

Special Issue

Severe Dry Eye With Combined Mechanisms is Involved in the Ocular Sequelae of SJS/TEN at the Chronic Stage

Chie Sotozono, Mayumi Ueta, and Norihiko Yokoi

Department of Ophthalmology, Kyoto Prefectural University of Medicine, Kyoto, Japan

Correspondence: Chie Sotozono, Department of Ophthalmology, Kyoto Prefectural University of Medicine, 465 Kajii-cho, Hirokoji-agaru, Kawaramachi-dori, Kamigyo-ku, Kyoto 602-0841, Japan; csotozon@koto.kpu-m.ac.jp.

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Stevens-Johnson syndrome (SJS), and its more severe variant, toxic epidermal necrolysis (TEN), are life-threatening diseases of the skin and mucous membranes. After the acute-stage damage subsides, serious visual impairment and severe dry eye remains as ocular sequelae. At the acute stage, ocular complications occur in 77% of SJS/TEN patients. In cases with pseudomembranous formation and/or epithelial defects, the risk of ocular sequelae increases. Among 13 slit-lamp microscopy images that we obtained of SJS/TEN patients at the chronic stage, the loss of corneal epithelial stem cells and severe meibomian gland involvement were found to be the most common disorders. Severe dry eye in SJS includes three important mechanisms: (1) aqueous tear deficiency, (2) decreased wettability of corneal surface, and (3) increased evaporation. Dry eye severity in SJS patients is often underestimated when the meniscus is first observed, as the punctum is closed due to scarring or surgery. In SJS patients with severe dry eye, the dryness results in immense eye pain, and unstable tear film related to dry eye result in a change/loss of vision. For the treatment of dry eye in SJS, it is important to suppress chronic inflammation on the ocular surface, and 2% rebamipide ophthalmic solution reportedly helps to obtain ocular surface stabilization. Scleral contact lenses, as well as the newly developed limbal-rigid contact lenses, improve the patients' visual acuity and reduce symptoms related to severe dry eye. Further studies and new therapeutic methods are needed to more effectively treat dry eye in patients afflicted with SJS/TEN.

Keywords: Stevens-Johnson syndrome, tear deficiency dry eye, meibomian gland dysfunction, evaporative dry eye, limbal rigid contact lens

Stevens-Johnson syndrome (SJS) and its more severe variant, toxic epidermal necrolysis (TEN), are acute inflammatory diseases of the skin and mucous membranes that predispose patients to life-threatening complications such as sepsis, respiratory dysfunction, and multi-organ failure.[1–4] Although the incidence of SJS and TEN is very low, both can affect anyone at any age, usually as a consequence of adverse drug reactions. After the acute-stage impairments subside, cicatrization of the ocular surface progresses.[5,6] Serious visual impairment and ocular discomfort arise, and continue throughout the patient's life.[7–9] Moreover, in cases of SJS and TEN, dry eye is the most frequent ocular sequelae.[10–12]

It should be noted that these diseases are severity variants of a single entity.[13,14] The classification is based on the clinical appearance during the "acute stage."[15] Briefly, a diagnosis of SJS is defined as when the skin detachment is less than 10% of the body surface area, and a diagnosis of TEN is defined as when over 30% of the body surface area is involved. A diagnosis of "overlapping SJS-TEN" is defined as when the detachment involves 10% to 30% of the body surface area. However, when patients are seen by ophthalmologists at the chronic stage, it is often difficult to distinguish between SJS and TEN, as many years have already passed since resolution of the dermatological changes. Ocular findings at the chronic stage in SJS are identical to those in TEN.[16] Thus, ocular surface diseases arising from SJS or TEN are collectively regarded as "SJS" from the ophthalmologist's perspective.

Clinical Features at the Acute Stage and Ocular Sequelae

In 77% of SJS/TEN patients, ocular complications are involved at the acute stage of the disease.[10] Bilateral acute conjunctivitis is known to occur prior to, or simultaneously with, acute fever and systemic eruption.[9] Extensive inflammation arises on the ocular surface with extreme upregulation of inflammatory cytokines, accompanied by pseudo-membranous formation and corneal and/or conjunctival epithelial defects.

Recently, we proposed a simple grading system to evaluate acute ocular severity of SJS/TEN, with the grades ranging from 0 to 3 based on the presence of conjunctivitis, corneal or conjunctival (ocular surface) epithelial defect, and pseudomembrane formation.[10] Bilateral conjunctival hyperemia was assessed as grade 1. Eyes with pseudomembrane formation or an ocular-surface epithelial defect were assessed as grade 2. Eyes with both pseudomembrane formation and an ocular surface epithelial defect were assessed as grade 3. In both SJS and TEN, the chronic ocular sequelae more frequently occur in patients with severe, or very severe, ocular involvement (grades 2 and 3 of the acute ocular severity score) than in patients with no or mild ocular involvement (grades 0 and 1). We found that the prevalence of dry eye at the chronic stage increases according to the increase of acute ocular severity (SJS: $P = 0.001$; TEN: $P = 0.014$; Fig. 1).[10]

We consider ocular surface inflammation and epithelial necrosis or apoptosis to be the initial ocular pathologic

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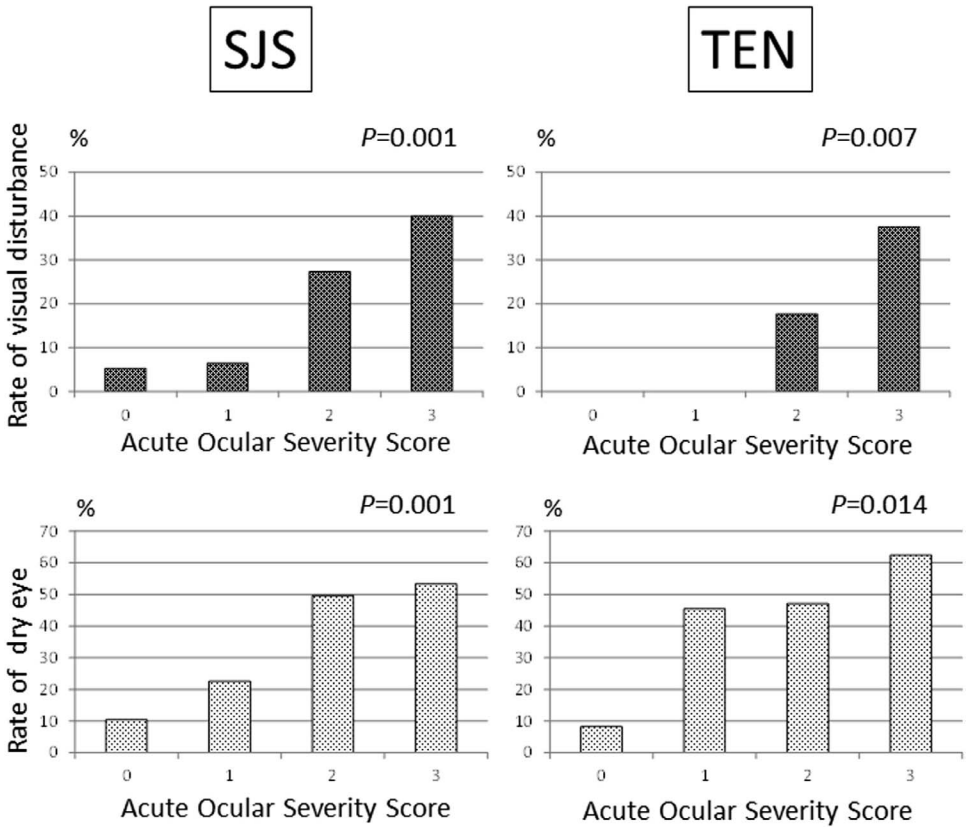

Figure 1. Relationship between the Acute Ocular Severity Score and ocular sequelae in SJS and TEN. The percentage rate of the presence of visual disturbance (top two charts) and eye dryness (bottom two charts) in the SJS and TEN cases, respectively, compared to the Acute Ocular Severity Score. The Cochran-Armitage Test was used to determine whether or not acute ocular severity was related to ocular sequelae.

processes of SJS/TEN. Secondary processes include persistent epithelial defects, ulceration and perforation, fornix shortening, symblepharon formation, and vision loss. Both SJS and TEN are self-limited diseases, and the systemic condition improves within 2 months after the onset. However, in cases with prolonged ocular surface inflammation, the secondary process can progress even after the systemic findings subside.

Objective Findings at the Chronic Stage

Previously, we developed a new grading system to evaluate chronic ocular complications in SJS/TEN.[8] In our new grading system, corneal complications (i.e., superficial punctate keratopathy, epithelial defect, loss of the palisades of Vogt [POV], conjunctivalization, neovascularization, opacification, and keratinization), conjunctival complications (i.e., hyperemia and symblepharon formation), and eyelid complications (i.e., trichiasis, mucocutaneous junction involvement, meibomian gland involvement, and punctal damage) were graded on a scale from 0 to 3 according to their severity (Fig. 2). The severity of meibomian gland involvement was determined clinically by the decreased quantity of the meibomian gland secretion expressed manually at the central-third of the upper lid margin. Of the above-described complications, the loss of the POV and severe meibomian gland involvement were most common ocular complications at the chronic stage (i.e., 82.6% and 73.9%, respectively).

In SJS/TEN cases, limbal stem cell destruction, evidenced by the loss of the POV, may occur at disease onset, thus resulting in conjunctivalization, neovascularization, and opacification of the cornea. Meibomian glands may also be involved in the injury after the onset of SJS. In addition, the ocular pathologic process is often accompanied by the destruction of goblet cells. Since the meibomian glands and goblet cells play a crucial role in tear-film stabilization, this is likely to contribute to the evaporative effect of dry eye via the instability of tear film. In the clinical setting, it is important to first understand that both "aqueous-tear-deficient" and "evaporative" dry eye are involved in the ocular sequelae of SJS/TEN at the chronic stage.

Meibomian Gland Dysfunction in SJS

The differences of meniscus tear volume, the condition of the precorneal tear film, and the structure of the meibomian glands in eyes with severe ocular surface disorders and in normal healthy eyes are shown in Figure 3. The study involved 69 eyes of 37 cases with SJS (mean age: 47.1 ± 21.3 [SD] years), 32 eyes of 17 cases with ocular cicatricial pemphigoid (OCP; mean age: 63.6 ± 18.9 years), 22 eyes of 16 cases with chemical/thermal injury (mean age: 42.6 ± 15.8 years), and 42 eyes of normal healthy control subjects (mean age: 49.3 ± 20.5 years). The meniscus tear volume was evaluated by measurement of the tear meniscus radius (TMR) via video meniscometry.[17,18] For evaluation of the pre-corneal tear film condition, a video-interferometer (DR-1; Kowa, Tokyo, Japan) was used to observe the specular image of the reflected light from the tear-film lipid layer (TFLL) at the central part of the cornea (2 mm circular area), with the images being graded from 1 to 5 based on our previously reported novel grading system.[19,20]

Using a video-meibography system,[21] the structure of the meibomian glands was evaluated from the point of the gland

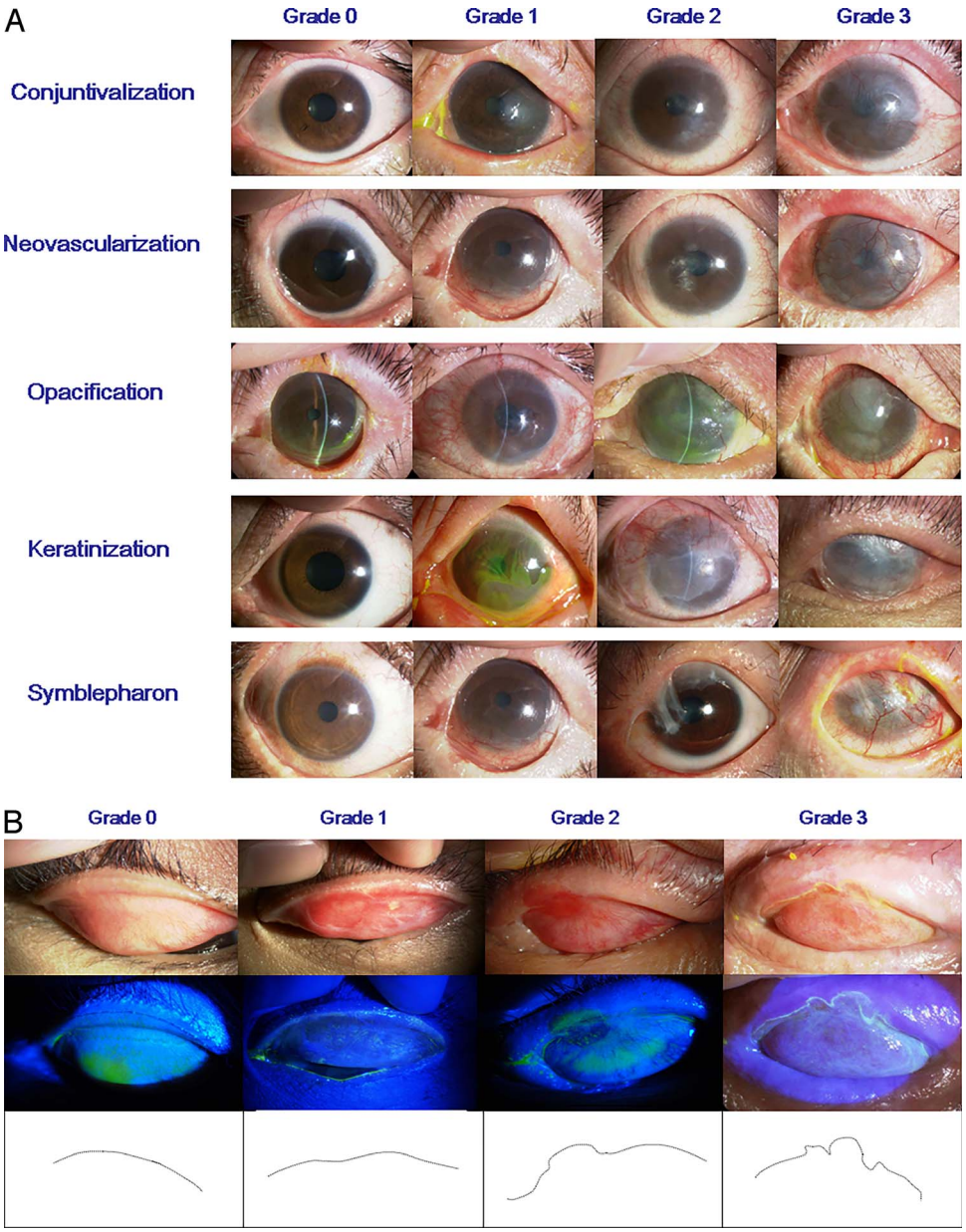

FIGURE 2. Ocular surface grading scores at chronic stage in SJS/TEN (**A**) Grading scores of corneal and conjunctival complications. (**B**) Grading scores of mucocutaneous junction involvement.

dropout of the meibomian glands of the lower eyelid, which was classified into one of the following 3 grades: 1) normal, 2) mild dropout, and 3) severe dropout.

Our findings revealed that the TMR values, as evaluated by meniscometry, did not differ among the 4 groups (i.e., 0.30 ± 0.26 mm in the SJS group, 0.25 ± 0.20 mm in the OCP group, 0.27 ± 0.18 mm in the chemical/thermal injury group, and 0.26 ± 0.19 mm in the normal healthy control group). On the other hand, the mean (±SD) grades of the TFLL, in which greater grades imply an abnormal tear film, were significantly greater in the SJS (4.1 ± 1.2; ±SD) and OCP (3.7 ± 1.1) groups than in the chemical/thermal injury (2.5 ± 1.0) and normal healthy control (2.3 ± 0.5) groups ($P < 0.05$). From these findings, it is important to note that among the 63 SJS eyes in which the TFLL could be examined, 31 eyes demonstrated a grade 5, the corneal condition uncovered by the complete tear film. This finding implies that in those SJS cases with grade 5, although the tear volume was maintained and compatible with that of normal controls, sufficient aqueous tears were not reflected upon the corneal surface, being uncovered by the tear film. This discrepancy might possibly be explained by the abnormal corneal surface in SJS patients. Grade 5 signifies the decrease of wettability of the epithelium; less water holding capacity of epithelium resulting in the decreased thickness of aqueous tear film on the surface of the cornea leading to the arrest of spreading of TFLL. In SJS eyes, it is reported that abnormal epithelial differentiation such as keratinization occurs on the ocular surface epithelium.[22] Taking into consideration the fact that normal corneal epithelium is known to have remarkably high wettability,[23] abnormal epithelial differentiation in SJS may result in a decreased wettability of the corneal surface, thus leading to an inability to establish complete precorneal tear film (grade 5).

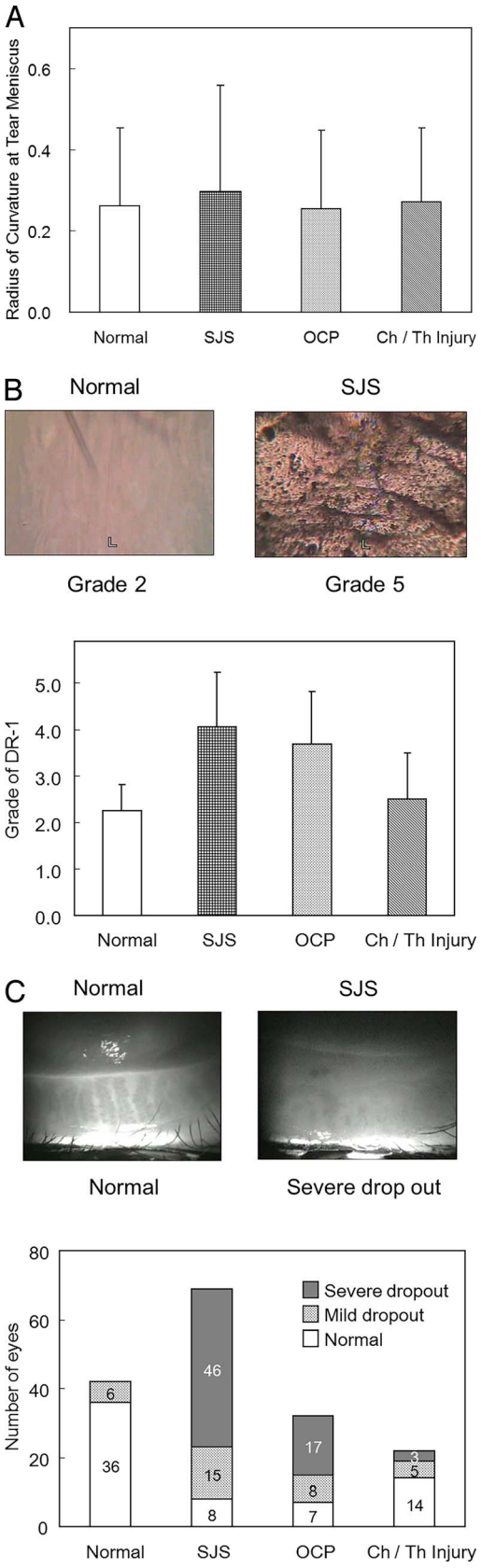

Figure 3. Differences of meniscus tear volume, condition of precorneal tear film, and the structure of meibomian glands in eyes with severe ocular surface disorders and normal healthy eyes. (**A**) TMR via the use of a video meniscometer. (**B**) The condition of precorneal tear film using a video-interferometer. (**C**) The structure of the meibomian glands evaluated by a video-meibography system.

Video-meibography showed high rates of severe dropout in meibomian glands in SJS and OCP (i.e., 66.7% and 53.1%, respectively). Since, severe meibomian gland dropout is known to be related to evaporative dry eye via the dysfunction of meibomian glands, the evaporative mechanism in SJS and OCP is also thought to be involved in the associated dry eye.

When considering the possible association of aqueous tear deficiency in SJS due to the involvement of the lacrimal gland duct in subconjunctival scarring, the results described above suggest that in SJS at the chronic phase, three important mechanisms are likely to be involved, (i.e., aqueous tear deficiency, decreased wettability due to corneal surface change via squamous metaplasia/keratinization, and increased evaporation due to meibomian gland dysfunction).

Subjective Symptoms Related to Dry Eye in SJS

Severe dryness of the ocular surface causes eye pain, foreign body sensation, photophobia, and visual disturbance, and patients often experience difficulty in opening their eyes. Sometimes, SJS patients present complaining of severe dryness of the eye despite frequent instillation of artificial tears. Thus, it is important to understand that severe dry eye with the combined mechanisms of aqueous tear deficiency, decreased corneal surface wettability, and increased evaporation may be involved in cicatrized cases with SJS. In eyes in which the superior and/or inferior punctum is closed due to scarring or surgery (e.g., punctal plugs or cauterization), tear deficiency can often be underestimated when the meniscus is first observed. SJS complications such as trichiasis, cicatricial entropion, and scarring of the mucocutaneous junction (lid margin) can be the cause of blink-related microtrauma, and enhance the symptoms related to dry eye.[7,8,11,12]

It should be noted that a stable tear film over the cornea is needed for consistent good vision, and that unstable tear film related to dry eye can result in a fluctuation of vision. In fact, SJS patients at the chronic stage often complain of fluctuating visual acuity, especially in the eye with severe dry eye, depending on the time after blinking. Using the functional visual acuity (FVA) measurement system, dynamic visual changes can be continuously measured under a 30-second blink-free period in one eye. Kaido et al.[24] examined the dynamic visual changes in SJS using the FVA system and reported that the time-related decline of FVA was greater in patients with SJS compared to normal subjects. In addition, the visual maintenance ratio (VMR) was found to be markedly lower in patients with SJS compared to patients with Sjögren syndrome (SS) and controls.[25]

Treatment for Dry Eye in SJS

For the proper treatment of dry eye in SJS cases, it is vital to pay attention to not only dry eye, but also to ocular surface inflammation. In SJS eyes at the chronic stage, persistent conjunctival inflammation exists.[26] Recently, we discovered chronic inflammation in the follicles of eyelashes.[27] To increase tear volume, the administration of artificial tears is necessary, and preservative-free artificial tears are recommended. Moreover, autologous serum/plasma is widely used for the treatment of dry eye or persistent corneal epithelial defects.[28–30] The use of a punctal plug or surgical punctal occlusion is also effective.[31] Low-dose topical steroids decrease the patients' symptoms. However, when topical steroids are used, strict attention should be paid to adverse events such as infectious keratitis and the elevation of intraocular pressure.

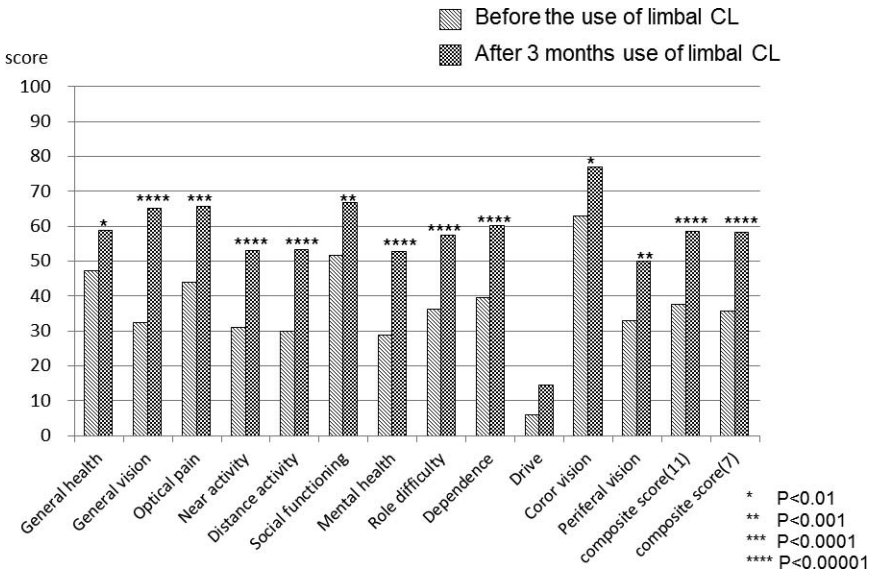

Figure 4. National Eye Institute Visual Function Questionnaire-25 of the patients with SJS or TEN before and after 3-months of limbal contact lens use.

It should be noted that 2% rebamipide ophthalmic suspension, which was specifically developed for the treatment of dry eye, suppresses inflammatory cytokine production in human corneal or conjunctival epithelial cells.[32–34] In a multicenter, open-label, 52-week study, five cases of SJS were included, and rebamipide was found to be safe and effective for SJS dry eye.[35,36] In that study, ocular surface stabilization was surprisingly obtained without steroids after the use of 2% rebamipide. Thus, it is highly possible that rebamipide ophthalmic suspension works to suppress ocular surface inflammation. In fact, in many SJS cases, we have successfully switched treatment from topical steroids to 2% rebamipide ophthalmic solution.

Recent reports have demonstrated the therapeutic benefits of scleral contact lenses (CLs) in the management of severe ocular surface diseases such as SJS and TEN.[37,38] However, scleral CLs are too large to use for severely cicatrized eyes with conjunctival fornix shortening. A newly developed limbal rigid CL (limbal CLs) with a 13.0- or 14.0-mm diameter size can be used in eyes with a short fornix and/or symblepharon.[39] Limbal CLs allow for tear exchange under the CL at the time of blinking, and their use improves the patients' visual acuity and reduces symptoms related to severe dry eye. Due to the decrease of tear evaporation, and possible improvement of wettability of the corneal surface covered by the limbal CL, eye pain dramatically decreases during limbal CL wear. It should be noted that the use of limbal CLs has been found to improve the patient's general health and overall wellbeing (Fig. 4).

As a surgical treatment for severe ocular disorders, amniotic membrane transplantation in the acute phase works to prevent late complications.[40] Moreover, oral mucus membrane grafts can be used for fornix reconstruction and tarsal conjunctival scarring.[41,42] Minor salivary glands transplantation was found to improve dry eye in SJS.[42] We also developed autologous cultivated oral mucosal epithelial transplantation (COMET). COMET is a novel therapeutic method that is used for reconstruction of the ocular surface in eyes afflicted with severe conjunctivalization, keratinization, and symblepharon.[43,44] The use of limbal CLs after COMET promises both the improvement of visual acuity and the decrease of dry eye symptoms in severely affected SJS eyes.[39]

Future Directions

At the acute stage, ocular involvement is often easily overlooked because of high mortality rates associated with serious systemic diseases. Thus, strict attention must be paid to ocular involvement at the acute stage. In cases treated with steroid pulse and intensive topical betamethasone at the early stage post disease onset, none of the eyes showed ocular sequelae.[45] Hence, recognition of ocular involvement at the acute stage of SJS/TEN may reduce the rate of ocular sequelae. Although a variety of ophthalmic solutions are currently available, and scleral CLs or limbal CLs are known to effectively reduce dry-eye-related symptoms, it is still difficult to treat and manage dry eye in SJS/TEN patients. Thus, further studies and new therapeutic methods are needed to more effectively treat dry eye in patients with SJS/TEN.

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Investigative Ophthalmology & Visual Science

JAMA Neurology | Original Investigation

Association of HLA-A*31:01 Screening With the Incidence of Carbamazepine-Induced Cutaneous Adverse Reactions in a Japanese Population

Taisei Mushiroda, PhD; Yukitoshi Takahashi, MD, PhD; Teiichi Onuma, MD, PhD; Yoshiaki Yamamoto, PhD; Tetsumasa Kamei, MD; Tohru Hoshida, MD; Katsuya Takeuchi, MD, PhD; Kotaro Otsuka, MD, PhD; Mitsutoshi Okazaki, MD, PhD; Masako Watanabe, MD, PhD; Kosuke Kanemoto, MD, PhD; Tomohiro Oshima, MD, PhD; Atsushi Watanabe, MD, PhD; Shiro Minami, MD, PhD; Kayoko Saito, MD, PhD; Hisashi Tanii, MD, PhD; Yasushi Shimo, MD, PhD; Minoru Hara, MD; Shinji Saitoh, MD, PhD; Toshihiko Kinoshita, MD, PhD; Masaki Kato, MD, PhD; Naoto Yamada, MD, PhD; Naoki Akamatsu, MD, PhD; Toshihiko Fukuchi, MD; Shigenobu Ishida, MD; Shingo Yasumoto, MD, PhD; Atsushi Takahashi, PhD; Takeshi Ozeki, PhD; Takahisa Furuta, MD, PhD; Yoshiro Saito, PhD; Nobuyuki Izumida, MEcon; Yoko Kano, MD, PhD; Tetsuo Shiohara, MD, PhD; Michiaki Kubo, MD, PhD; for the GENCAT Study Group

IMPORTANCE Carbamazepine, a commonly used antiepileptic drug, is one of the most common causes of cutaneous adverse drug reactions (cADRs) worldwide. The allele HLA-A*31:01 is reportedly associated with carbamazepine-induced cADRs in Japanese and European populations; however, the clinical utility of HLA-A*31:01 has not been evaluated.

OBJECTIVE To assess the use of HLA-A*31:01 genetic screening to identify Japanese individuals at risk of carbamazepine-induced cADRs.

DESIGN, SETTING, AND PARTICIPANTS This cohort study was conducted across 36 hospitals in Japan from January 2012 to November 2014 among 1202 patients who had been deemed suitable to start treatment with carbamazepine. Preemptive HLA-A*31:01 genetic screening was performed for 1187 participants. Patients who did not start treatment with carbamazepine or alternative drugs were excluded. Participants were interviewed once weekly for 8 weeks to monitor the development of cADRs. Data analysis was performed from June 8, 2015, to December 27, 2016.

EXPOSURES Neuropsychiatrists were asked to prescribe carbamazepine for patients who tested negative for HLA-A*31:01 and alternative drugs for those who tested positive for HLA-A*31:01.

MAIN OUTCOMES AND MEASURES Incidence of carbamazepine-induced cADRs.

RESULTS Of the 1130 included patients who were prescribed carbamazepine or alternative drugs, the mean (range) age was 37.4 (0-95) years, 614 (54.3%) were men, and 198 (17.5%) were positive for HLA-A*31:01. Expert dermatologists identified 23 patients (2.0%) who had carbamazepine-induced cADRs, of which 4 patients required hospitalization. Drug-induced hypersensitivity syndrome was observed for 3 patients, maculopapular eruption for 9 patients, erythema multiforme for 5 patients, and an undetermined type of cADR for 6 patients. No patient developed Stevens-Johnson syndrome or toxic epidermal necrolysis. Compared with historical controls, the incidence of carbamazepine-induced cADRs was significantly decreased (for BioBank Japan data: incidence, 3.4%; odds ratio, 0.60; 95% CI, 0.36-1.00; $P = .048$; for the Japan Medical Data Centre claims database: incidence, 5.1%; odds ratio, 0.39; 95% CI, 0.26-0.59; $P < .001$).

CONCLUSIONS AND RELEVANCE Preemptive HLA-A*31:01 genetic screening significantly decreased the incidence of carbamazepine-induced cADRs among Japanese patients, which suggests that it may be warranted in routine clinical practice.

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Editorial
Supplemental content

Author Affiliations: Author affiliations are listed at the end of this article.

Group Information: The research staff members at the institutions comprising the Genotype-Based Carbamazepine Therapy (GENCAT) study group appear at the end of this article.

Corresponding Author: Michiaki Kubo, MD, PhD, RIKEN Center for Integrative Medical Sciences, 1-7-22, Suehiro-cho, Tsurumi-ku, Yokohama 230-0045, Japan (michiaki.kubo@riken.jp).

Cutaneous adverse drug reactions (cADRs) are independent of the dose prescribed, are unpredictable, and are sometimes life-threatening.[1] Several drugs are known to cause higher incidences of cADRs.[1] Although recent advances in pharmacogenomics have shown the importance of genetic variations in cADRs induced by several drugs,[2-6] the clinical implementation of such pharmacogenomic findings has been slow, mainly owing to the need to establish the clinical utility of the genetic variations for decisions regarding drug prescriptions.[7]

Many patients of European ancestry have experienced cADRs after filling carbamazepine prescriptions, with the frequency varying from 3.7% to 13%.[8-11] For carbamazepine-induced cADRs, the allele HLA-B*15:02 was first reported to be associated with Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) in a Han Chinese population.[12-14] Prospective genetic screening has revealed the clinical utility of HLA-B*15:02.[15] The association of HLA-A*31:01 with carbamazepine-induced cADRs has also been reported in Japanese[16] and European[10] populations: the risk of HLA-A*31:01 was 8.0 times as high as that among tolerant controls in a Japanese population with mild cADRs and 33.9 times as high for SJS/TEN in the same population.[16] Similar trends were observed in the European population.[10] However, to date, the clinical utility of HLA-A*31:01 has not yet been evaluated.

In Japan, indications for the use of carbamazepine have not changed since 2003, and no description of the utility of genetic testing has been incorporated into the carbamazepine label in contrast to these labels in the United States and Taiwan. Therefore, to examine the clinical utility of HLA-A*31:01, we conducted a genotype-based carbamazepine therapy (GENCAT) study to determine whether screening for HLA-A*31:01 prior to prescribing carbamazepine reduces the incidence of carbamazepine-induced cADRs.

Key Points

Question Does an association exist between genetic screening for the allele HLA-A*31:01 and reduced incidence of carbamazepine-induced cutaneous adverse drug reactions among Japanese patients?

Findings In this cohort study conducted in Japan, neuropsychiatrists were asked to prescribe carbamazepine for HLA-A*31:01–negative patients and alternative drugs for HLA-A*31:01–positive patients. Of the 1130 patients, 23 (2.0%) had carbamazepine-induced cutaneous adverse drug reactions, a significant 40% decrease compared with the incidence observed in a historical control group.

Meaning Preemptive HLA-A*31:01 screenings might reduce the rate of carbamazepine-induced cutaneous adverse drug reactions in routine clinical practice, providing additional evidence for implementing individualized medicine.

Methods

Study Design

This study was registered with the University Hospital Medical Information Network Clinical Trials Registry of Japan before enrollment began. We recruited and enrolled patients from 36 cooperating hospitals throughout Japan from January 2012 to November 2014. To ensure that all hospitals complied with regulations and protocol requirements, the study was monitored by Sogo Rinsho Medefi. Patients who fulfilled the following 3 criteria were invited to participate: (1) those deemed suitable to start treatment with carbamazepine based on the decision of neuropsychiatrists at cooperating hospitals, (2) those of Japanese descent of any age who had not received carbamazepine within 1 month of enrollment, and (3) those patients (or guardians) providing written informed consent. We excluded patients with a history of carbamazepine-induced cADRs, patients who were or were planning to become pregnant, and patients with renal failure (serum creatinine level ≥2.5 mg/dL [to convert to micromoles per liter, multiply by 88.4]), liver cirrhosis, or hypoproteinemia (serum albumin level ≤2.5 g/dL [to convert to grams per liter, multiply by 10]). All severe adverse reactions were monitored by an efficacy and safety evaluation committee (T. Furuta, Y. Saito, and N.I.). We performed the study in accordance with the provisions of the Declaration of Helsinki.[17] The study protocol was approved by the research ethics committees of RIKEN, all cooperating hospitals, and the Institute of Medical Science, University of Tokyo. All participants or their guardians provided written informed consent.

We obtained a sample (2 mL) of whole blood from each participant. The HLA-A*31:01 screening was performed at participating hospitals using an automated molecular diagnostic system.[18,19] The HLA-A*31:01 status was reported within 1.5 hours to the neuropsychiatrists (including Y.T., T. Onuma, T. Kamei, T.H., K.T., K.O., M.O., M.W., K.K., T. Oshima, A.W., S.M., K.S., H.T., Y. Shimo, M.H., S.S., T. Kinoshita, M. Kato, N.Y., N.A., T. Fukuchi, S.I., and S.Y.), who explained the HLA-A*31:01 screening result and the risk of carbamazepine-induced cADRs to participants or guardians. Patients who were negative for HLA-A*31:01 were recommended a prescription of carbamazepine, whereas those who were positive for HLA-A*31:01 were prescribed alternative drugs according to the neuropsychiatrists' recommendations. Telephone interviews of all participants were conducted once weekly for 8 weeks to monitor for symptoms of cADRs or until the discontinuation of prescribed drugs owing to adverse drug reactions or miscellaneous causes. Other adverse events were graded according to the Common Terminology Criteria for Adverse Events Version 3.0 from the National Cancer Institute. This follow-up length was selected because most carbamazepine-induced cADRs occur within 2 months of the start of treatment with carbamazepine.[15]

At enrollment, participants were requested to immediately visit cooperating hospitals or the nearest hospital specializing in dermatology for evaluation of any suspected cADR symptoms. We asked the neuropsychiatrists to start appropriate treatment immediately following the initial diagnosis and to provide detailed case reports of the cADRs by completing a standardized case report form that included onset, symptoms, treatment, outcome, cADR photographs, and the initial diagnosis provided by the attending dermatologist. The col-

lected case reports were independently reviewed by 2 expert dermatologists (Y.K. and T.S.) who classified the carbamazepine-induced cADR diagnoses into the following 4 categories: definite, meaning that there was sufficient information to diagnose carbamazepine-induced cADRs; probable, meaning that some information was missing but that the collected information was sufficient to diagnose carbamazepine-induced cADRs; possible, meaning that the reported cADR indicated a possibility of other skin disease or cADR caused by other drugs; and unlikely, meaning that the patients were considered to have developed other skin diseases or cADRs caused by other drugs or that information was insufficient. To determine the cADR culprit drug, we considered a cADR to be caused by another drug if the drug treatment was started or terminated during the carbamazepine treatment and if the cADR onset date was within 1 week of the start of the other drug treatment. Other drug treatments that were started during the carbamazepine treatment and continued even after the cADR resolved were excluded. Detailed classification criteria are given in eTable 1 in the Supplement. In this study, we defined only definite or probable cases as carbamazepine-induced cADRs. The 2 expert dermatologists (Y.K. and T.S.) also classified cADRs into an SJS/TEN, drug-induced hypersensitivity syndrome (DIHS), a drug reaction with eosinophilia and systemic symptoms (DRESS) syndrome, a maculopapular eruption, an erythema multiforme, a fixed drug eruption, and so on. If the collected information was insufficient, the cADR was defined as an undetermined type. If the diagnosis of the 2 expert dermatologists was discordant, they achieved a final diagnosis following discussion.

Genetic Screening for HLA-A*31:01

We previously developed a rapid genotyping method to detect the presence or absence of HLA-A*31:01.[19] This method was applied to a DNA chip developed by RIKEN Genesis, which monitored the HLA-A*31:01 screening performed at cooperating hospitals throughout the study.

Validation of HLA-A*31:01 Genetic Screening

To examine the accuracy of HLA-A*31:01 screening, we collected an additional sample (5 mL) of whole blood from participants at enrollment. The whole blood was sent to a central laboratory (BML Inc) for DNA extraction. Anonymized DNA samples were transferred to and stored at BioBank Japan.

For analytical validation, we used DNA samples stored at BioBank Japan and genotyped HLA-A alleles using a WAKFlow HLA typing kit (Wakunaga), which is based on a Luminex system. Data analysis was performed with WAKFlow typing software using HLA sequence–specific oligonucleotide probes and a reverse line blot assay (Dynal Biotech). We found that the HLA-A*31:01 genetic screening results were wholly consistent with the results obtained using the Luminex system for all samples analyzed.

Historical Incidence

BioBank Japan is a multi-institutional hospital-based registry that was established in 2003 to collect genomic DNA and clinical information from Japanese patients who received a diagnosis of any of 47 diseases, including epilepsy and drug eruption.[20,21] Physicians at cooperating hospitals provide the diagnoses. Clinical information is collected at baseline and then annually after the baseline survey through reviews of medical records using a standardized questionnaire. We searched the BioBank Japan clinical database from April 1, 2003, to December 28, 2010, and found 1274 patients who were prescribed carbamazepine and 44 patients who experienced a carbamazepine-induced cADR. A carbamazepine-induced cADR was identified as a diagnosis of drug eruption with carbamazepine specified as the culprit drug or as an adverse drug reaction, such as intoxication dermatosis or drug eruption, with carbamazepine specified as a culprit drug. Six patients were included in the group with 1274 patients prescribed carbamazepine and in the group of 44 patients who experienced a carbamazepine-induced cADR. Thus, we calculated the incidence of carbamazepine-induced cADRs as 44 to be 1312 carbamazepine users (3.4%) over the course of the study.

We also searched the Japan Medical Data Centre (JMDC) claims database[22] from January 1, 2005, to December 31, 2014. Carbamazepine-induced cADRs were defined by the presence of at least 1 of the following *International Statistical Classification of Diseases and Related Health Problems, Tenth Revision (ICD-10)* codes during the period coincident with a prescription of carbamazepine: drug-induced dermatitis (*ICD-10* codes L27.0, L27.1, and L27.9), erythema multiforme, including SJS/TEN (*ICD-10* code L51), drug urticaria (*ICD-10* code L50.8), carbamazepine intoxication (*ICD-10* code T42.1), adverse reaction caused by antiepileptic drugs (*ICD-10* code T42.6), and drug hypersensitivity (*ICD-10* code T88.7). We found 12 060 patients who were prescribed carbamazepine, 610 of whom experienced a cADR, for an incidence of carbamazepine-induced cADRs of 5.1%.

Statistical Analysis

We analyzed all patients who received carbamazepine or alternative medications after HLA-A*31:01 screening. Analyses were conducted with SAS, version 9.2 (SAS Institute Inc) from June 8, 2015, to December 27, 2016. We used the Fisher exact test to compare the incidence of carbamazepine-induced cADRs with historical incidences. To evaluate the significance of any differences in clinical characteristics, we used 2-tailed, unpaired *t* tests or 2-tailed χ^2 tests as appropriate. All reported *P* values are 2-sided, and $P < .05$ was considered statistically significant. On the basis of the incidence of carbamazepine-induced cADRs obtained using BioBank Japan data, we determined that 1059 participants would provide a statistical power of 80% to detect a reduction in the incidence from 3.4% to 1.5% with a 2-sided significance level of .05.

Results

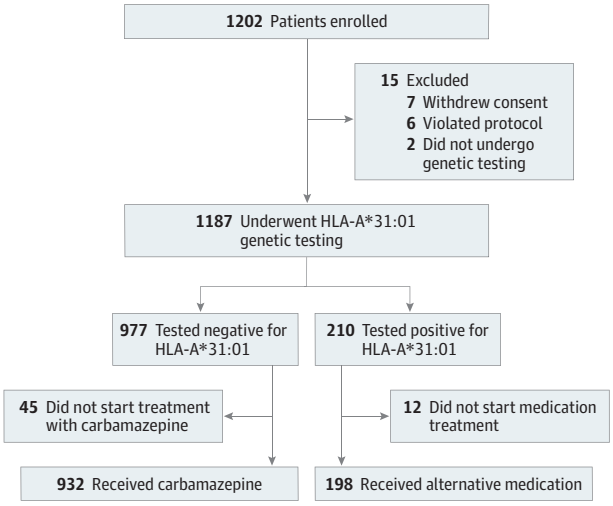
Study Participants

The **Figure** shows the participant flowchart for this GENCAT study. Of the 1202 enrolled participants, 1187 underwent preemptive HLA-A*31:01 screening. Of the 210 participants positive for HLA-A*31:01, we excluded 12 because the neuropsy-

chiatrists decided not to start any medication treatment. Of the 977 participants negative for HLA-A*31:01, we excluded 45 who did not start treatment with carbamazepine. Thus, 1130 patients were included in the analysis. All 932 patients negative for HLA-A*31:01 started carbamazepine treatment. The 198 patients positive for HLA-A*31:01 were prescribed various alternative drugs (eTable 2 in the Supplement). There were 9 patients who had been prescribed drugs other than carbamazepine before the enrollment; these patients continued using the same drugs without adding alternative drugs because the neuropsychiatrists determined that no other drug was more appropriate. Despite the screening result, 1 patient positive for HLA-A*31:01 started carbamazepine treatment based on the neuropsychiatrist's decision.

Table 1 provides the demographic and clinical characteristics of the study participants. The mean age was 37.4 years (range, 0-95 years), and 614 men were included. The indications for carbamazepine included epilepsy, schizophrenia, bipolar disorder, and trigeminal neuralgia. There were no differences in mean age, sex distribution, or the indication for carbamazepine between the patients who were positive and the patient who were negative for HLA-A*31:01.

Figure. Flowchart of Screened Patients



Adverse Events

During the 8-week follow-up, discontinuation of carbamazepine treatment because of cADRs, other adverse reactions, or other causes of carbamazepine occurred among 153 patients who tested negative for HLA-A*31:01, and discontinuation of alternative drugs occurred among 19 patients who tested positive for HLA-A*31:01. No significant difference was observed in the frequency of carbamazepine or alternative medication discontinuation due to cADRs between patients who tested negative (43 of 932 [4.6%]) and patients who tested positive (4 of 198 [2.0%]) for HLA-A*31:01 (P = .12).

After the expert review, among the 43 patients who were negative for HLA-A*31:01, carbamazepine-induced cADRs were diagnosed as being definite for 11 patients, probable for 12 patients, possible for 9 patients, and unlikely for 11 patients (eTable 3 in the Supplement). Of the 4 HLA-A*31:01-positive patients with cADRs, 3 had cADRs caused by other drugs, and 1 had insufficient information. Although 1 HLA-A*31:01-positive patient was prescribed carbamazepine, this patient did not develop a cADR.

Definite or probable carbamazepine-induced cADRs were observed in 23 patients (2.0%) in this study (**Table 2**). No patient developed SJS/TEN. Of the 23 patients with a definite or probable carbamazepine-induced cADR, 4 (2 with DIHS or DRESS, 1 with a maculopapular eruption, and 1 with erythema multiforme) required hospitalization for treatment. The 2 patients with DIHS or DRESS and the patient with a maculopapular eruption underwent intravenous steroid pulse therapy, whereas the patient with an erythema multiforme recovered following the discontinuation of carbamazepine treatment.

We also monitored patients for other adverse events, including fever, sore throat, fatigue, dizziness, insomnia, and gastrointestinal symptoms. The frequency of these adverse events showed no significant differences between HLA-A*31:01-positive and HLA-A*31:01-negative patients (eTable 4 in the Supplement).

Incidence of Carbamazepine-Induced cADRs

When we compared the incidence of carbamazepine-induced cADRs observed in this study with that determined for the historical controls obtained using data from BioBank

Table 1. Demographic and Clinical Characteristics of Participants Grouped by HLA-A*31:01 Genetic Screening Results

Characteristic	Participants, No. (%)		*P* Value
	Negative for HLA-A*31:01 (n = 932)	Positive for HLA (n = 198)	
Sex			
Male	510 (54.7)	104 (52.5)	.58
Female	422 (45.3)	94 (47.5)	
Age, mean (range), y	37.1 (0-95)	38.5 (0-89)	.44
Indication for carbamazepine[a]			
Epilepsy	737 (78.5)	151 (75.9)	.25
Schizophrenia	55 (5.9)	9 (4.5)	
Bipolar disorder	38 (4.0)	15 (7.5)	
Trigeminal neuralgia	42 (4.5)	11 (5.5)	
Other condition	67 (7.1)	13 (6.5)	

[a] Eight patients had multiple diagnoses.

Japan, the results of the present study showed a significant decrease in the incidence of carbamazepine-induced cADRs of nearly 40% (odds ratio, 0.60; 95% CI, 0.36-1.00; $P = .048$) (**Table 3**). Using the JMDC claims database as an independent historical control, we also found a significant decrease in the incidence of carbamazepine-induced cADRs in the present study (odds ratio, 0.39; 95% CI, 0.26-0.59; $P < .001$) (Table 3). When we limited the cases to the 932 HLA-A*31:01–negative patients who were exposed to carbamazepine, the incidence of carbamazepine-induced cADRs (2.5%) did not significantly differ from that of the BioBank Japan (odds ratio, 0.73; 95% CI, 0.44-1.22; $P = .26$) but was significantly lower than that of the JMDC database (odds ratio, 0.48; 95% CI, 0.31-0.72; $P < .001$).

Discussion

In this prospective cohort study using HLA-A*31:01 screening prior to treatment, 1130 patients were prescribed carbamazepine or alternative drugs on the basis of the genetic screening results. Among them, 23 patients developed carbamazepine-induced cADRs during the 8-week follow-up. Comparison with a historical control indicated that the preemptive use of HLA-A*31:01 genetic screening in the present study was associated with a 40% reduction in the incidence of carbamazepine-induced cADRs. The frequency of HLA-A*31:01 carriers in the population was high (17.7%) in this study, although previous reports have also indicated a similar expected frequency of HLA-A*31:01 carriers (16.6%-17.5%).[23,24] These results suggested that HLA-A*31:01 genetic screening would be useful for the prevention of carbamazepine-induced cADRs among Japanese patients.

For carbamazepine-induced cADRs, the clinical utility of the HLA-B*15:02 genetic test has already been established by preemptive screening,[15] and the test is recommended in the Clinical Pharmacogenetics Implementation Consortium guidelines.[25] However, the frequency of the HLA-B*15:02 allele is low in Korean, Japanese, African, and European populations.[23] In addition, HLA-B*15:02 is specifically associated with SJS/TEN. By contrast, HLA-A*31:01 is associated with carbamazepine-induced cADRs in Japanese,[16,26,27]

Table 2. Expert Dermatologist Diagnoses Following Their Review of Cases Initially Reported as Cutaneous Adverse Drug Reactions Irrespective of Causative Drug

	Patients, No.						
	Positive for HLA-A*31:01[b]		Negative for HLA-A*31:01				
Adverse Event[a]	Possible	Unlikely	Definite	Probable	Possible	Unlikely	Total
Drug-induced hypersensitivity syndrome	0	0	2	1	0	0	3
Maculopapular eruption	0	2	5	4	4	1	16
Erythema multiforme	0	0	4	1	2	0	7
Other	0	0	0	0	0	4	4
Unknown	1	1	0	6	3	6	17
Total	1	3	11	12	9	11	47

[a] No patient developed Stevens-Johnson syndrome, toxic epidermal necrolysis, or fixed drug eruption.

[b] No HLA-A*31:01–positive patient developed a definite or probably adverse event.

Table 3. GENCAT Study and Historical Control Incidences of Carbamazepine-Induced Cutaneous Adverse Reactions

	Patients, No.		
		Historical Control	
Adverse Event	GENCAT Study (N = 1130)	BioBank Japan Data (N = 1312)	JMDC Claims Database (N = 12 060)
Carbamazepine-induced cutaneous adverse reaction,[a] No. (%)	23 (2.0)	44 (3.4)	610 (5.1)
Stevens-Johnson syndrome or toxic epidermal necrolysis	0	3	6
Drug-induced hypersensitivity syndrome	3	1	NA
Maculopapular eruption	9	6	NA
Erythema multiforme	5	15	NA
Fixed drug eruption	0	0	NA
Others	0	8	NA
Unknown	6	11	NA
Statistical analysis, compared with GENCAT study			NA
P value		.048	<.001
Odds ratio (95% CI)		0.60 (0.36-1.0)	0.39 (0.26-0.59)

Abbreviations: GENCAT, Genotype-Based Carbamazepine Therapy; JMDC, Japan Medical Data Centre; NA, not available, regarding information on specific cutaneous adverse drug reactions.

[a] In the GENCAT study, all 23 patients who developed definite or probable carbamazepine-induced cutaneous adverse reactions were negative for HLA-A*31:01.

Han Chinese,[13] Northern European,[10] Korean,[28] and Canadian[29] populations. The frequency of the HLA-A*31:01 allele is 7% to 9% in Japanese, 5% in Korean, 2% in Chinese, 2% to 3% in European, and 1% in African populations.[23] Moreover, HLA-A*31:01 has been associated with a full spectrum of carbamazepine-induced cADRs. Therefore, HLA-A*31:01 genetic screening prior to prescribing carbamazepine would be useful for preventing many types of carbamazepine-induced cADRs in a range of patient populations.

Similar to studies evaluating HLA-B*15:02 genetic screening, no patient in the present study developed SJS/TEN. We compared the incidence of SJS/TEN in this study with those of 2 historical controls (BioBank Japan: incidence, 0.23%; $P = .25$; JMDC database: incidence, 0.05%; $P > .99$) (Table 3); however, we found no significant differences, likely because the present study was designed to evaluate the association of HLA-A*31:01 with all types of carbamazepine-induced cADRs.

Our study showed that HLA-A*31:01 screening was associated with a significant reduction in the incidence of carbamazepine-induced cADRs; however, a 2.0% incidence of carbamazepine-induced cADRs still remained. This indicates that even patients who tested negative for HLA-A*31:01 should be monitored for cADRs. In our previous study,[16] genetic screening of HLA-A*31:01 had a sensitivity of 60.7% and a specificity of 87.5%, which suggested that some HLA-A*31:01-positive individuals will not develop cADRs. In the present study, neuropsychiatrists could not find suitable alternative drugs for 9 patients who tested positive for HLA-A*31:01. Moreover, 1 patient who tested positive for HLA-A*31:01 was prescribed carbamazepine and did not develop cADRs. Thus, although the results of the present study indicate that drug alternatives to carbamazepine are recommended for treating patients with HLA-A*31:01, neuropsychiatrists may prescribe carbamazepine after considering both the patient's genetic risk of developing a cADR and the risk of withholding the drug. Further research to identify additional genetic factors is needed for a more precise anticipation of carbamazepine-induced cADRs.

In addition to the clinical utility, the cost-effectiveness of HLA-A*31:01 screening should be discussed. The debate on the implementation of genetic testing has arisen in part because of the uncertainty as to whether it is truly cost-effective for the health care system when accounting for the cost of testing, increased cost of alternative treatments, and low incidence of cADRs. A recent cost-effectiveness analysis conducted in a UK health care setting showed that HLA-A*31:01 testing reduced the expected rate of cADRs from 780 to 700 per 10 000 patients with an incremental cost-effective ratio of £12 808 (approximately US $17 700) per quality-adjusted life-years.[30] Because the UK National Institute for Health and Care Excellence specifies an incremental cost-effective ratio of £20 000 (approximately US $27 660) as the threshold to represent cost-effective use of resources by the National Health Service in the United Kingdom,[31] routine testing for HLA-A*31:01 is likely to be cost-effective. However, because health insurance systems differ for each country, an economic evaluation should be conducted from the perspective of the national health insurance system and the clinical settings of Japan.

Limitations

The primary limitation of this study was its nonrandomized study design. However, the clinical utility of HLA alleles associated with cADRs has been shown by a prospective randomized study[32] and single-arm prospective screening studies.[15,33] The presence of HLA-A*31:01 has been associated with increased risk of all types of cADRs and has substantially increased the risk of life-threatening SJS/TEN.[10,16] Moreover, cADRs occasionally progress after discontinuation of carbamazepine treatment.[1] Therefore, ethical considerations disallowed a randomized study design. To help mitigate this limitation, we compared the incidence of carbamazepine-induced cADRs with that of historical controls. Because there was no reliable information available on a Japanese population, we first used data from BioBank Japan. Case ascertainment of a carbamazepine-induced cADR in the present study was equivalent to that of Biobank Japan. However, the incidence of carbamazepine-induced cADR may have been underestimated in this historical control because some mild cases might not have been reported in the patients' medical records. Therefore, we also accessed the JMDC claims database, the largest epidemiology claims database available in Japan. The definition of a cADR in the JMDC database is broader than that used for BioBank Japan because of the lack of culprit drug information. Hence, we included cADR cases caused by other drugs and unlikely cases due to insufficient information as cADR cases for comparison with those in the JMDC historical control. We found that the incidence of carbamazepine-induced cADRs was still significantly decreased with the use of preemptive HLA-A*31:01 genetic screening in the present study (2.8% vs 5.1%, $P<.001$). Because the prevalence of carbamazepine-induced cADRs has been reported to be between 3.7% and 13%,[8-11] we believe the results of the present study were not distorted by an overestimation of historical controls.

A second limitation is that we did not include genetic testing for HLA-B*15:02, a well-known genetic predictor of a carbamazepine-induced cADR. However, the carrier frequency of HLA-B*15:02 is estimated to be 0.062%[24]; thus, the expected number of HLA-B*15:02 carriers in our study would have been less than 1 patient. In addition, the aim of the present study was to evaluate the clinical utility of HLA-A*31:01. Thus, we believe the lack of HLA-B*15:02 genetic testing did not affect our findings. A third limitation is that although we excluded patients who took carbamazepine within 1 month of study entry, we did not exclude patients who had previously been exposed to carbamazepine without a history of a cADR. However, inclusion of those patients in this study would have decreased the incidence of cADRs regardless of genotype.

Conclusions

Preemptive HLA-A*31:01 screening prior to dispensing carbamazepine or alternative drug prescriptions was associated with a 40% reduction in the incidence of cADRs in a Japanese population compared with a historical control.

Although cost-effectiveness analyses are required, the use of HLA-A*31:01 screening to reduce the rate of carbamazepine-induced cADRs in routine clinical practice appears warranted.

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Author Affiliations: RIKEN Center for Integrative Medical Sciences, Yokohama, Japan (Mushiroda, A. Takahashi, Ozeki, Kubo); Department of Clinical Research, National Epilepsy Center, Shizuoka Institute of Epilepsy and Neurological Disorders, Shizuoka, Japan (Y. Takahashi, Yamamoto); Musashino-Kokubunji Clinic, Tokyo, Japan (Onuma); Tokushukai Hospital, Tokyo, Japan (Kamei); National Hospital Organization Nara Medical Center, Nara, Japan (Hoshida); Department of Neuropsychiatry, Iwate Medical University, Iwate, Japan (Takeuchi, Otsuka); Kitariasu Hospital, Iwate, Japan (Takeuchi); Department of Psychiatry, National Center of Neurology and Psychiatry, Tokyo, Japan (Okazaki, M. Watanabe); Neuropsychiatric Department, Aichi Medical University, Aichi, Japan (Kanemoto, Oshima); Division of Personalized Genetic Medicine, Nippon Medical School, Tokyo, Japan (A. Watanabe); Institute for Advanced Medical Sciences, Nippon Medical School, Kawasaki, Japan (Minami); Institute of Medical Genetics, Tokyo Women's Medical University, Tokyo, Japan (K. Saito); Department of Psychiatry, Mie University Graduate School of Medicine, Mie, Japan (Tanii); Department of Neurology, Juntendo University School of Medicine, Tokyo, Japan (Shimo); Hara Clinic, Yokohama, Japan (Hara); Department of Pediatrics and Neonatology, Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan (Saitoh); Department of Neuropsychiatry, Kansai Medical University, Osaka, Japan (Kinoshita, Kato); Department of Psychiatry, Shiga University of Medical Science, Shiga, Japan (Yamada); Department of Neurology, University of Occupational and Environmental Health, Kitakyusyu, Japan (Akamatsu); Suzukake Clinic, Nagoya, Japan (Fukuchi); Department of Neuropsychiatry, Kurume University School of Medicine, Fukuoka, Japan (Ishida, Yasumoto); Center for Clinical Research, Hamamatsu University School of Medicine, Hamamatsu, Japan (Furuta); Division of Medicinal Safety Science, National Institute of Health Sciences, Tokyo, Japan (Y. Saito); Department of Empirical Social Security Research, National Institute of Population and Social Security Research, Tokyo, Japan (Izumida); Department of Dermatology, Kyorin University School of Medicine, Tokyo, Japan (Kano, Shiohara).

Author Contributions: Drs Mushiroda and Kubo 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.
Study concept and design: Mushiroda, Onuma, Otsuka, Okazaki, M. Watanabe, Kanemoto, A. Watanabe, Minami, Kinoshita, Ishida, Ozeki, Kano, Kubo.
Acquisition, analysis, or interpretation of data: Mushiroda, Y. Takahashi, Yamamoto, Kamei, Hoshida, Takeuchi, Otsuka, Okazaki, M. Watanabe, Kanemoto, Oshima, A. Watanabe, Minami, K. Saito, Tanii, Shimo, Hara, Saitoh, Kato, Yamada, Akamatsu, Fukuchi, Yasumoto, A. Takahashi, Ozeki, Furuta, Y. Saito, Izumida, Shiohara, Kubo.
Drafting of the manuscript: Mushiroda, Y. Takahashi, Onuma, Hoshida, Takeuchi, Otsuka, Oshima, K. Saito, Tanii, Hara, Kinoshita, Ishida, Kano, Kubo.
Critical revision of the manuscript for important intellectual content: Mushiroda, Yamamoto, Kamei, Otsuka, Okazaki, M. Watanabe, Kanemoto, A. Watanabe, Minami, Shimo, Hara, Saitoh, Kato, Yamada, Akamatsu, Fukuchi, Yasumoto, A. Takahashi, Ozeki, Furuta, Y. Saito, Izumida, Shiohara, Kubo.
Statistical analysis: Mushiroda, Otsuka, A. Takahashi, Kubo.
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Study supervision: Otsuka, Okazaki, M. Watanabe, Furuta, Kubo.

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Group Information: The Genotype-Based Carbamazepine Therapy (GENCAT) study group comprises research staff members at the following cooperating hospitals: Juntendo University School of Medicine (Juntendo University Hospital, Juntendo University Urayasu Hospital, Juntendo University Shizuoka Hospital; Yasushi Shimo, MD, PhD), Nippon Medical School Hospital (Atsushi Watanabe, MD, PhD), Nippon Medical School Musashi Kosugi Hospital (Shiro Minami, MD, PhD), Iwate Medical University Hospital (Katsuya Takeuchi, MD, PhD; Kotaro Otsuka, MD, PhD), Tokushukai Hospital (Uji Tokushukai Hospital, Kishiwada Tokushukai Hospital, Sapporo Tokushukai Hospital, Shonan Kamakura General Hospital, Shonan Fujisawa Tokushukai Hospital, Chiba Tokushukai Hospital, Chiba-Nishi General Hospital, Chubu Tokushukai Hospital, Fukuoka Tokushukai Hospital, Nozaki Tokushukai Hospital, Tokyo Nishi Tokushukai Hospital; Tetsumasa Kamei, MD), Shiga University of Medical Science Hospital (Naoto Yamada, MD, PhD), Tokyo Women's Medical University Hospital (Kayoko Saito, MD, PhD), Aichi Medical University Hospital (Kosuke Kanemoto, MD, PhD), Aichi Medical University Medical Clinic (Kosuke Kanemoto, MD, PhD), Suzukake Clinic (Toshihiko Fukuchi, MD), National Center Hospital, National Center of Neurology and Psychiatry (Mitsutoshi Okazaki, MD, PhD), Nagoya City University Hospital (Shinji Saitoh, MD, PhD), Hospital of the University of Occupational and Environmental Health (Naoki Akamatsu, MD, PhD), Kansai Medical University Hospital (Toshihiko Kinoshita, MD, PhD), Kansai Medical University Medical Center (Toshihiko Kinoshita, MD, PhD), Musashino-Kokubunji Clinic (Teiichi Onuma, MD, PhD), National Hospital Organization Nara Medical Center (Tohru Hoshida, MD), Kurume University Hospital (Shigenobu Ishida, MD; Shingo Yasumoto, MD, PhD), Mie University Hospital (Hisashi Tanii, MD, PhD), National Mie Hospital (Hisashi Tanii, MD, PhD), Shizuoka Institute of Epilepsy and Neurological Disorders (Yukitoshi Takahashi, MD, PhD), and the Hara Clinic (Minoru Hara, MD).

Additional Contributions: Hidenori Sugano MD, PhD, Department of Neurosurgery, Juntendo University School of Medicine, collected clinical data and invited participants to take part in the study. Koichi Matsuda, MD, PhD, Kaori Muto, PhD, Hyunsoo Hong, PhD, Yasushi Yamashita, and Yusuke Nishikubo with The Institute of Medical Science at The University of Tokyo supported the biobanking and provided ethical discussions for the GENCAT study. We thank all the patients who participated in this study and the research staff at the cooperating hospitals. No one received financial compensation for the stated contribution.

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