161: 115-120, 2009.

3. Kimura H, Miyake K, Yamauchi Y, Nishiyama K, Iwata S, Iwatsuki K, Gotoh K, Kojima S, Ito Y, Nishiyama Y: Idetification of epstein-barr virus (EBV)-infected lymphocyte subtypes by flow cytometric in situ hybridization in EBV-associated lymphoproliferative diseases. J Infectious Deseases 200: 1078-1087, 2009.

4. Suzuki R, Suzumiya J, Yamaguchi M, Nakamura S, Kameoka J, Kojima H, Abe M, Kinoshita T, Yoshino T, Iwatsuki K, Kagami Y, Tsuzuki T, Kurokawa M, Ito K, Kawa K, Oshimi K for the NK-cell Tumor Study Group: Prognostic factors for mature natural killer (NK) cell neoplasms: aggressive NK cell leukemia and extranodal NK cell lymphoma, nasal type. Annals of Oncology, Epib, 2009.

5. Tanaka N, Dainichi T, Ohyama B, Yasumoto S, Oono T, Iwatsuki K, Elfert S, Fritsch A, Brunckner-Tuderman L, Hashimoto T: A case of epidermolysis bullosa acquisita with clinical features of Brunsting-Perry pemphigoid showing an excellent response to colchicine. J Am Acad Dermatol 61:715-719, 2009.

6. Senoh A, Tokuyama Y, Nakayama Y, Fujii K, Iwatsuki K.: Erythema multiforme- like contact reaction due to liquid- formulated 2,2-dibrome-3- nitrilopropionamide: involvement of cytotoxic. Clin Exp Dermatol 34; e732-736, 2009.

7. Sugiyama-Fukamatsu H, Suzuki N, Nakanishi G, Iwatsuki K: Epidermolysis bullosa nevus arising in a patient with Dowling-Meara type epidermolysis bullosa simplex with a novel K5 mutation. J Dermatol 36: 447-452, 2009.

8. Nakanishi G, Fujii K, Asagoe K, Iwatsuki K: Human papillomavirus genome integration in multifocal vulvar Bowen's disease and squamous cell carcinoma. Clin Exp Dermatol 34:e965-967, 2009.

9. 岩月啓氏、照井　正、小澤　明、小宮根真弓、梅澤慶紀、中西　元、原　弘之、馬渕智生、青山裕美、北島康雄。膿疱性乾癬（汎発型）診療ガイドライン2009年度版。http://www.dermatol.or.jp/medical/guideline/pdf/nouhou_kansen.pdf. 簡易版日皮会誌掲載予定

学会発表

1. Iwatsuki K.: Internaional Society for Cutaneous Lymphomas Annual Meeting, San Francisco, U.S.A., March 5, 2009.

2. Iwatsuki K.: Cutaneous disorders associated with Epstein-Barr virus-associated NK/T lymphoproliferative disorders: Cellular and molecular events. (Symposia) The 4 th Joint Meeting of Japanese Dermatological Association and Australasian College of Dermatologists, Sapporo, Japan, July 10-12, 2009.

3. Iwatsuki K: Cutaneous disorders associated with Epstein-Barr virus infections. 35th Annual Meeting of the Taiwanese Dermatological Association, Taipei, Taiwan, November 21-22, 2009.

H. 知的所有権の出願・登録状況（予定を含む）

なし。

I. 引用文献

1. Zenz R, Eferl R, Kenner L, Florin L, Hummerich L, Mehic D, et al. Psoriasis-like skin disease and arthritis caused by inducible epidermal deletion of Jun proteins. Nature 2005;437:369-75.
2. Roth J, Vogl T, Sorg C, Sunderkotter C. Phagocyte-specific S100 proteins: A novel group of proinflammatory molecules. Trends Immunol 2003;24:155-8.
3. Nukui T, Ehama R, Sakaguchi M, Sonegawa H, Katagiri C, Hibino T, et al. S100A8/A9, a key mediator for positive feedback growth stimulation of normal human keratinocytes. J Cell Biochem 2008;104:453-64.
4. Benoit S, Toksoy A, Ahlmann M, Schmidt M, Sunderkotter C, Foell D, et al. Elevated serum levels of calcium-binding S100 proteins A8 and A9 reflect disease activity and abnormal differentiation of keratinocytes in psoriasis. Br J Dermatol 2006;155:62-6.

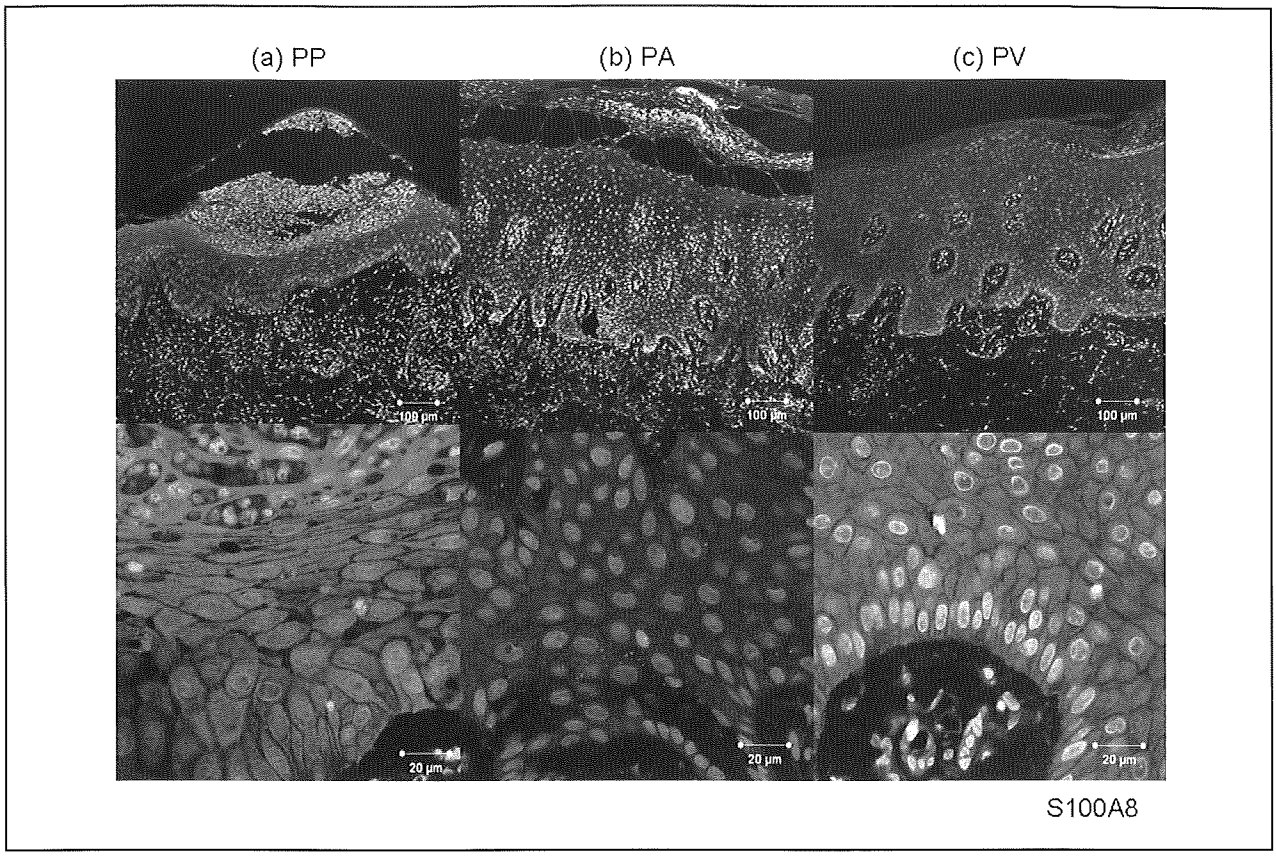

図１．乾癬病変部におけるS100A8発現

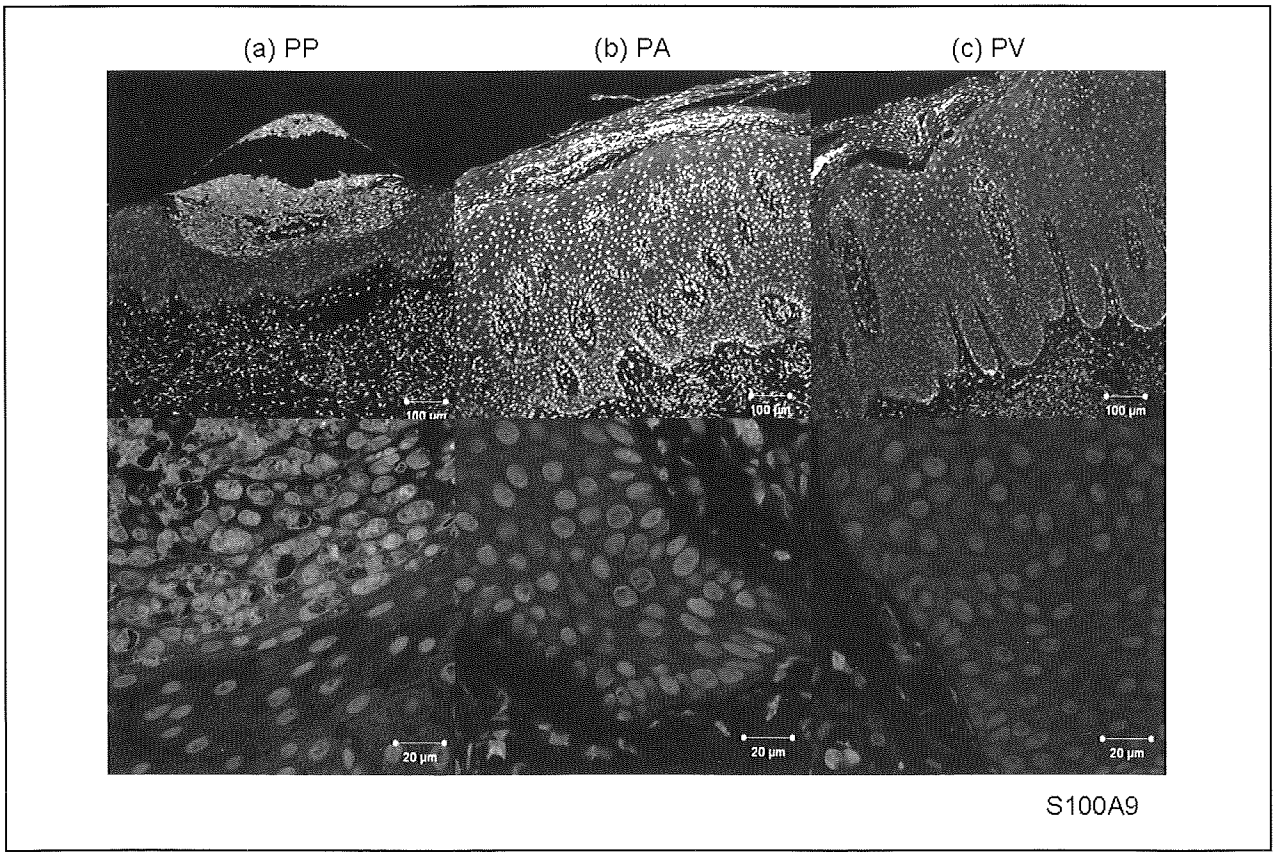

図２．乾癬病変部のS100A9発現

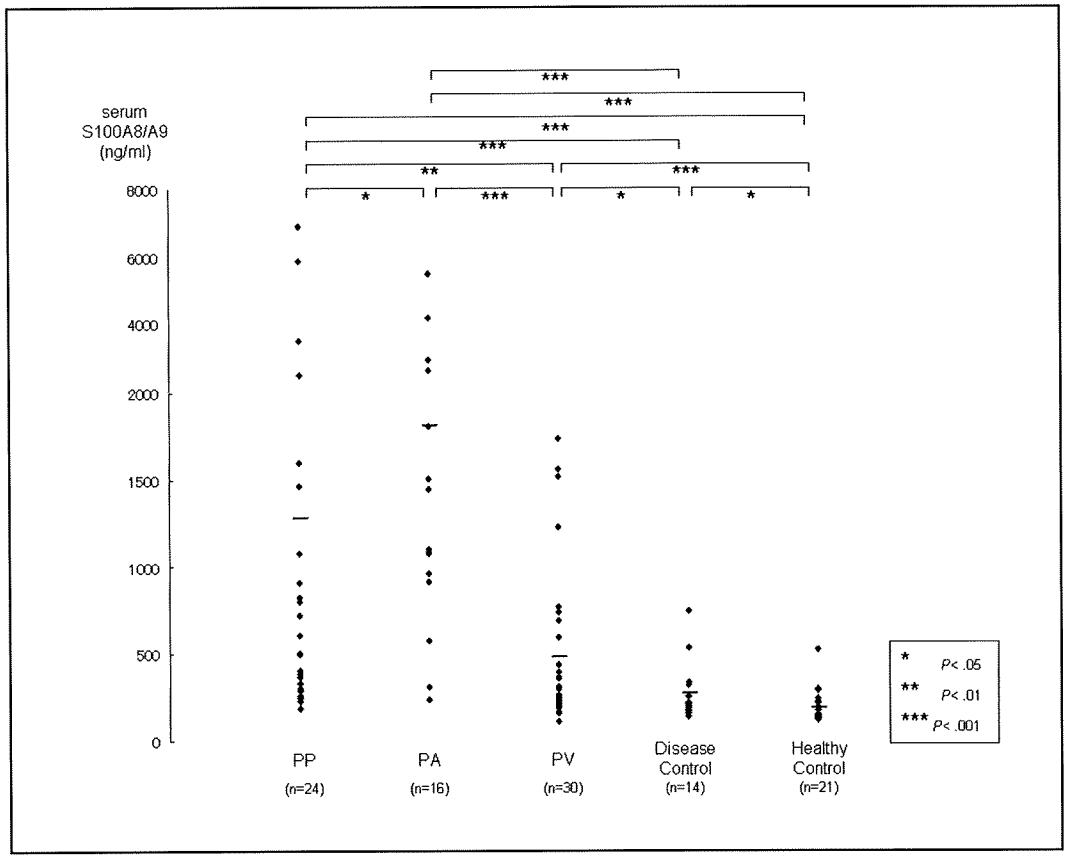

図３．乾癬病型別血清S100A8/A9　レベル

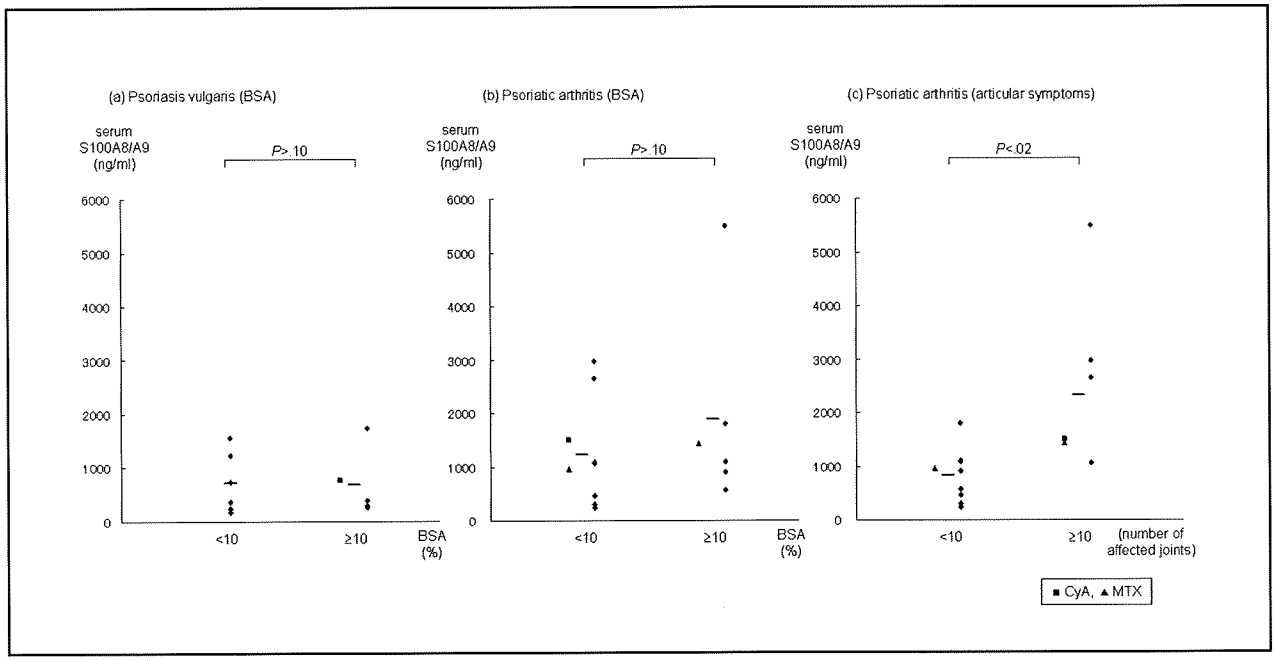

図４．血清S100A８/A９と皮疹重症度および関節症状の関連

厚生労働科学研究費補助金（難治性疾患克服研究事業）
分担研究報告書

幽門閉鎖合併単純型表皮水疱症におけるプレクチン発現パターンの解析

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研究要旨　プレクチンはプラキンファミリーに属するクロスリンカー蛋白であり、皮膚や筋肉などの様々な臓器で発現している。プレクチンは、両端のglobular domainにrod domainが挟まれるダンベル状の構造を有しており、筋ジストロフィー合併単純型表皮水疱症（EBS-MD）患者のほとんどは、プレクチンのrod domainをコードする*PLEC1*のexon31に遺伝子変異を認める。これに対して幽門閉鎖合併単純型表皮水疱症（EBS-PA）患者においては大部分がexon31以外に遺伝子変異がみられる。今回われわれは、EBS-PA患者の皮膚組織、培養細胞におけるプレクチンの発現パターンを解析した。EBS-MDでは、全長のプレクチンを発現せずにrod domainが欠損した蛋白（rodless）の発現が保たれているという特徴を有していたが、EBS-PAでは全長のプレクチン、rodlessともに発現していなかった。

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A．研究目的

プレクチンは、中央のrod domainをN末端とC末端のglobular domainがそれぞれ挟み込む、独特なダンベル様構造を呈する細胞骨格リンカー蛋白であり、皮膚・筋肉・消化管をはじめとした様々な臓器で発現している。プレクチンには全長の蛋白とともに、rod domainが除かれたrodlessプレクチンが選択的スプライシングによって生じることが知られている。

幽門閉鎖合併型表皮水疱症（EBS-PA）は、プレクチンをコードする*PLEC1*の遺伝子変異によって生じることが知られている。過去の報告では、EBS-PA患者の多くがrod domainをコードするエクソン31以外のエクソンで遺伝子変異を有するとされている。本研究の目的はプレクチンの様々な部位に対する抗体を用いることで、EBS-PA患者におけるプレクチンの詳細な発現パターンを解析し、病態解明につなげることである。

B．研究方法

プレクチンのEBS-PAの３症例について患者皮膚を解析した。プレクチンパターンを蛍光抗体法で解析した。プレクチンのN末端領域、rod領域、C末端領域のそれぞれに対する抗体を用いて、患者皮膚組織のプレクチンの発現パターンを明らかとした。また、EBS-PA患者由来羊水細胞のプレクチン発現パターンについて蛋白レベルとmRNAレベルで解析した。

C．研究結果

EBS-PA皮膚組織の蛍光抗体法の結果、プレクチンのN末端、rod domain、C末端の発現が著明に減弱～消失していた。免疫ブロットによる解析では、正常ヒト線維芽細胞抽出液で全長のプレクチンとrodlessプレクチンが発現していたが、EBS-PA由来羊水細胞抽出液では全長のプレクチン、rodlessプレクチンともに著明に減弱していた。mRNAレベルでもEBS-PA由来羊水細胞では全長とrodlessプレクチンをコードする転写産物が減少していた。

D．考　察

本研究の結果は、ほとんどのEBS-PAの患者で*PLEC1*変異がrod領域をコードするエクソン31以外のエクソンに認められるという報告と一致する。すなわち、*PLEC1*変異がrod領域以外にあるために、全長とrodlessプレクチンはともにnonsense-mediated mRNA decayによって発現が消失してしまうと考えられる。この結果は、EBS-MDにおいて全長のプレクチンは消失しているものの、rodlessプレクチンの発現が保たれているという所見とは対照的であり、このプレクチンの発現パターンの違いが、異なる臨床型を引き起こしていると考えられる。

E．結　論

EBS-PAでは全長、rodlessプレクチンともに著明に発現が減弱～消失している。

F．健康危険情報

特記すべきことなし。

G．研究発表（平成21年度）

論文発表

1．Abe R, Yoshioka N, Murata J, Fujita Y, Shimizu H:
Granulysin as a marker for early diagnosis of the Stevens-Johnson syndrome.
Ann Intern Med 151: 514-515, 2009.

2．Akiyama M, Sakai K, Hayasaka K, Tabata N, Yamada M, Ujiie H, Shibaki A, Shimizu H:
Conradi-Hunermann-Happle syndrome with abnormal lamellar granule contents.
Br J Dermatol 160: 1335-1337, 2009.

3．Amagai M, Ikeda S, Shimizu H, Iizuka H, Hanada K, Aiba S, Kaneko F, Izaki S, Tamaki K, Ikezawa Z, Takigawa M, Seishima M, Tanaka T, Miyachi Y, Katayama I, Horiguchi Y, Miyagawa S, Furukawa F, Iwatsuki K, Hide M, Tokura Y, Furue M, Hashimoto T, Ihn H, Fujiwara S, Nishikawa T, Ogawa H, Kitajima Y, Hashimoto K:
A randomized double-blind trial of intravenous immunoglobulin for pemphigus.
J Am Acad Dermatol 60: 595-603, 2009.

4．Ando S, Abe R, Sasaki M, Murata J, Inokuma D, Shimizu H:
Bone marrow-derived cells are not the origin of the cancer stem cells in ultraviolet-induced skin cancer.
Am J Pathol 174: 595-601, 2009.

5．Aoyagi S, Hata H, Iitani MM, Homma E, Inokuma D, Shimizu H:
Squamous cell carcinoma of the auricle with rhabdoid features.
J Cutan Pathol 36: 919-921, 2009.

6．Aoyagi S, Izumi K, Hata H, Kawasaki H, Shimizu H:
Usefulness of real-time tissue elastography for detecting lymph-node metastases in squamous cell carcinoma.
Clin Exp Dermatol 34: e744-e747, 2009.

7．Asaka T, Akiyama M, Domon T, Nishie W, Natsuga K, Fujita Y, Abe R, Kitagawa Y, Shimizu H:
Type XVII collagen is a key player in tooth enamel formation.
Am J Pathol 174: 91-100, 2009.

8．Asaka T, Akiyama M, Kitagawa Y, Shimizu H:
Higher density of label-retaining cells in gingival epithelium.
J Dermatol Sci 55: 132-134, 2009.

9．Bohgaki T, Atsumi T, Bohgaki M, Furusaki A, Kondo M, Sato-Matsumura KC, Abe R, Kataoka H, Hori-

ta T, Yasuda S, Amasaki Y, Nishio M, Sawada K, Shimizu H, Koike T:
Immunological reconstitution after autologous hematopoietic stem cell transplantation in patients with systemic sclerosis: relationship between clinical benefits and intensity of immunosuppression.
J Rheumatol 36: 1240-1248, 2009.

10. Goto-Ohguchi Y, Nishie W, Akiyama M, Tateishi Y, Aoyagi S, Tsuji-Abe Y, Sawamura D, Ishii N, Hashimoto T, Shimizu H:
A severe and refractory case of anti-p200 pemphigoid resulting in multiple skin ulcers and scar formation.
Dermatology 218: 265-271, 2009.

11. Hamasaka A, Abe R, Koyama Y, Yoshioka N, Fujita Y, Hoshina D, Sasaki M, Hirasawa T, Onodera S, Ohshima S, Leng L, Bucala R, Nishihira J, Shimizu T, Shimizu H:
DNA vaccination against macrophage migration inhibitory factor improves atopic dermatitis in murine models.
J Allergy Clin Immunol 124: 90-99, 2009.

12. Hamasaka E, Akiyama M, Hata H, Aoyagi S, Shimizu H:
Melanonychia caused by Stenotrophomonas maltophilia.
Clin Exp Dermatol 34: 242-243, 2009.

13. Honda A, Abe R, Yoshihisa Y, Makino T, Matsunaga K, Nishihira J, Shimizu H, Shimizu T:
Deficient deletion of apoptotic cells by macrophage migration inhibitory factor (MIF) overexpression accelerates photocarcinogenesis.
Carcinogenesis 30: 1597-1605, 2009.

14. Hsu CK, Akiyama M, Nemoto-Hasebe I, Nomura T, Sandilands A, Chao SC, Lee JY, Sheu HM, McLean WH, Shimizu H:
Analysis of Taiwanese ichthyosis vulgaris families further demonstrates differences in FLG mutations between European and Asian populations.
Br J Dermatol 161: 448-451, 2009.

15. Imada K, Dainichi T, Yokomizo A, Tsunoda T, Song YH, Nagasaki A, Sawamura D, Nishie W, Shimizu H, Fukagawa S, Urabe K, Furue M, Hashimoto T, Naito S:
Birt-Hogg-Dube syndrome with clear-cell and oncocytic renal tumour and trichoblastoma associated with a novel FLCN mutation.
Br J Dermatol 160: 1350-1353, 2009.

16. Inokuma D, Aoyagi S, Saito N, Iitani MM, Homma E, Hamasaka K, Shimizu H:
Bowen's disease of the nail matrix presenting as melanonychia: detection of human papillomavirus type 56.
Acta Derm Venereol 89: 638-639, 2009.

17. Inokuma D, Kodama K, Natsuga K, Kasai M, Abe M, Nishie W, Abe R, Hashimoto T, Shimizu H:
Autoantibodies against type XVII collagen C-terminal domain in a patient with bullous pemphigoid associated with psoriasis vulgaris.
Br J Dermatol 160: 451-454, 2009.

18. Inokuma D, Sawamura D, Shibaki A, Abe R, Shimizu H:
Scleroedema adultorum associated with sarcoidosis.
Clin Exp Dermatol 34: e428-429, 2009.

19. Inomata K, Aoto T, Binh NT, Okamoto N, Tanimura S, Wakayama T, Iseki S, Hara E, Masunaga T, Shimizu

H, Nishimura EK:
Genotoxic stress abrogates renewal of melanocyte stem cells by triggering their differentiation.
Cell 137: 1088-1099, 2009.

20. Ito K, Sawamura D, Goto M, Nakamura H, Nishie W, Sakai K, Natsuga K, Shinkuma S, Shibaki A, Uitto J, Denton CP, Nakajima O, Akiyama M, Shimizu H:
Keratinocyte-/fibroblast-targeted rescue of Col7a1-disrupted mice and generation of an exact dystrophic epidermolysis bullosa model using a human COL7A1 mutation.
Am J Pathol 175: 2508-2517, 2009.

21. Kanda M, Natsuga K, Nishie W, Akiyama M, Nagasaki A, Shimizu T, Shimizu H:
Morphological and genetic analysis of steatocystoma multiplex in an Asian family with pachyonychia congenita type 2 harbouring a KRT17 missense mutation.
Br J Dermatol 160: 465-468, 2009.

22. Kawabori M, Kuroda S, Nakayama N, Kenmotsu Y, Shimizu H, Tanino M, Iwasaki Y:
Spontaneous giant aneurysm of the superficial temporal artery: case report.
Neurol Med Chir (Tokyo) 49: 198-201, 2009.

23. Long HA, McMillan JR, Qiao H, Akiyama M, Shimizu H:
Current advances in gene therapy for the treatment of genodermatoses.
Curr Gene Ther 9: 487-494, 2009.

24. McMillan JR, Long HA, Akiyama M, Shimizu H, Kimble RM:
Epidermolysis bullosa (EB) - diagnosis and therapy.
Wound Practice and Research 17: 62-70, 2009.

25. Natsuga K, Shinkuma S, Nishie W, Shimizu H:
Animal models of epidermolysis bullosa.
Dermatol Clin 28: 137-142, 2009.

26. Nemoto-Hasebe I, Akiyama M, Kudo S, Ishiko A, Tanaka A, Arita K, Shimizu H:
Novel mutation p.Gly59Arg in GJB6 encoding connexin 30 underlies palmoplantar keratoderma with pseudoainhum, knuckle pads and hearing loss.
Br J Dermatol 161: 452-455, 2009.

27. Nemoto-Hasebe I, Akiyama M, Nomura T, Sandilands A, McLean WH, Shimizu H:
Clinical severity correlates with impaired barrier in filaggrin-related eczema.
J Invest Dermatol 129: 682-689, 2009.

28. Nemoto-Hasebe I, Akiyama M, Nomura T, Sandilands A, McLean WH, Shimizu H:
FLG mutation p.Lys4021X in the C-terminal imperfect filaggrin repeat in Japanese patients with atopic eczema.
Br J Dermatol 161: 1387-1390, 2009.

29. Nishie W, Sawamura D, Natsuga K, Shinkuma S, Goto M, Shibaki A, Ujiie H, Olasz E, Yancey KB, Shimizu H:
A novel humanized neonatal autoimmune blistering skin disease model induced by maternally transferred antibodies.
J Immunol 183: 4088-4093, 2009.

30. Nomura T, Akiyama M, Sandilands A, Nemoto-Hasebe I, Sakai K, Nagasaki A, Palmer CN, Smith FJ, McLean WH, Shimizu H:
Prevalent and rare mutations in the

gene encoding filaggrin in Japanese patients with ichthyosis vulgaris and atopic dermatitis.
J Invest Dermatol 129: 1302-1305, 2009.

31. Nomura Y, Abe M, Natsuga K, Moriuchi R, Kawasaki H, Mayuzumi M, Yasuoka A, Shimizu H:
Widespread keratosis follicularis squamosa.
Clin Exp Dermatol 34: 519-520, 2009.

32. Nomura Y, Akiyama M, Nishie W, Shimizu H:
Progressive Refractory Ulcer of the Nipple: A Quiz.
Acta Derm Venereol 89: 445-447, 2009.

33. Nomura Y, Nishie W, Shibaki A, Ibata M, Shimizu H:
Disseminated cutaneous Mycobacterium kansasii infection in an patient infected with the human immunodeficiency virus.
Clin Exp Dermatol 34: 625-626, 2009.

34. Osawa R, Akiyama M, Yamanaka Y, Ujiie H, Nemoto-Hasebe I, Takeda A, Yanagi T, Shimizu H:
A novel PTPN11 missense mutation in a patient with LEOPARD syndrome.
Br J Dermatol 161: 1202-1204, 2009.

35. Qiao H, Shibaki A, Long HA, Wang G, Li Q, Nishie W, Abe R, Akiyama M, Shimizu H, McMillan JR:
Collagen XVII participates in keratinocyte adhesion to collagen IV, and in p38MAPK-dependent migration and cell signaling.
J Invest Dermatol 129: 2288-2295, 2009.

36. Sakai K, Akiyama M, Yanagi T, McMillan JR, Suzuki T, Tsukamoto K, Sugiyama H, Hatano Y, Hayashitani M, Takamori K, Nakashima K, Shimizu H:
ABCA12 is a major causative gene for non-bullous congenital ichthyosiform erythroderma.
J Invest Dermatol 129: 2306-2309, 2009.

37. Shinkuma S, Abe R, Nishimura M, Natsuga K, Fujita Y, Nomura T, Nishie W, Shimizu H:
Secondary syphilis mimicking warts in an HIV-positive patient.
Sex Transm Infect 85: 484, 2009.

38. Shinkuma S, Akiyama M, Torii-Saito N, Natsuga K, Tateishi Y, Ito K, Hirota J, Shimizu Y, Shichinohe T, Shimizu H:
Pemphigus foliaceus associated with oesophageal cancer.
J Eur Acad Dermatol Venereol 23: 473-474, 2009.

39. Suzuki K, Yamaguchi Y, Villacorte M, Mihara K, Akiyama M, Shimizu H, Taketo MM, Nakagata N, Tsukiyama T, Yamaguchi TP, Birchmeier W, Kato S, Yamada G:
Embryonic hair follicle fate change by augmented {beta}-catenin through Shh and Bmp signaling.
Development 136: 367-372, 2009.

40. Uematsu R, Shinohara Y, Nakagawa H, Kurogochi M, Furukawa J, Miura Y, Akiyama M, Shimizu H, Nishimura S:
Glycosylation specific for adhesion molecules in epidermis and its receptor revealed by glycoform-focused reverse genomics.
Mol Cell Proteomics 8: 232-244, 2009.

41. Ujiie H, Akiyama M, Osawa R, Shida S, Aoyagi S, Shimizu H:

Bloody nipple discharge in an infant.
Arch Dermatol 145: 1068-1069, 2009.

42. Yanagi T, Shimizu T, Kodama K, Nemoto-Hasebe I, Kasai M, Shimizu H:
CD30-positive primary cutaneous anaplastic large-cell lymphoma and definite squamous cell carcinoma.
Clin Exp Dermatol 34: e293-294, 2009.

43. Ando S, Sato-Matsumura K, Kasai M, Nemoto-Hasebe I, Hoshina D, Ohyama B, Hashimoto T, Shimizu H:
Desmoglein1 and bullous pemphigoid 180 ELISA indexes correlating with disease activity in a patient with coexisting pemphigus foliaceus and bullous pemphigoid.
Clinical and Experimental Dermatology, in press.

44. Aoyagi S, Hata H, Homma E, Shimizu H:
Controlling the histological margin for non-melanoma skin cancer conveniently using a double-bladed scalpel.
J Surg Oncol, in press.

45. Iitani MM, Abe R, Yanagi T, Hamasaka A, Tateishi Y, Abe Y, Ito M, Kondo T, Kubota K, Shimizu H:
Aleukemic leukemia cutis with extensive bone involvement.
J Am Acad Dermatol, in press.

46. Inokuma D, Shibaki A, Shimizu H:
Two cases of cutaneous sporotrichosis in continental/ microthermal climate zone: global warming alert?
Clin Exp Dermatol, in press.

47. Kusajima E, Akiyama M, Sato M, Natsuga K, Shimizu H:
Type XVII collagen ELISA indices significantly decreased after bullous pemphigoid remission.
Int J Dermatol, in press.

48. Natsuga K, Nishie W, Akiyama M, Nakamura H, Shinkuma S, McMillan JR, Nagasaki A, Has C, Ouchi T, Ishiko A, Hirako Y, Owaribe K, Sawamura D, Bruckner-Tuderman L, Shimizu H:
Plectin expression patterns determine two distinct subtypes of epidermolysis bullosa simplex.
Hum Mutat, in press.

49. Natsuga K, Nishie W, Shinkuma S, Moriuchi R, Shibata M, Nishimura M, Hashimoto T, Shimizu H:
Circulating IgA and IgE autoantibodies in antilaminin-332 mucous membrane pemphigoid.
Br J Dermatol, in press.

50. Natsuga K, Sawamura D, Goto M, Homma E, Goto-Ohguchi Y, Aoyagi S, Akiyama M, Kuroyanagi Y, Shimizu H:
Response of intractable skin ulcers in recessive dystrophic epidermolysis bullosa patients to an allogeneic cultured dermal substitute.
Acta Derm Venereol, in press.

51. Ujiie H, Kodama K, Akiyama M, Shimizu H:
Hereditary benign telangiectasia: Two families with punctate telangiectasias surrounded by anemic halos.
Arch Dermatol, in press.

52. Ujiie H, Shibaki A, Akiyama M, Shimizu H:
Successful treatment of nail lichen planus with topical tacrolimus.
Acta Derm Venereol, in press.

53. Ujiie H, Shibaki A, Nishie W, Sawamura D, Wang G, Tateishi Y, Li Q, Moriuchi R, Qiao H, Nakamura H, Akiyama M, Shimizu H:
A novel active mouse model for bullous pemphigoid targeting humanized pathogenic antigen.
J Immunol, in press.

54. Vinzenz O, Gianluca T, Akiyama M, Claudine B-B, Christine B, Emmanuelle B, Philippe C, John JD, Peter E, Judith F, Philip F, Michal G, John H, Hashimoto T, Ingrid H, Hans CH, Daniel H, Alain H, Ishida-Yamamoto A, Withold KJ, Sancy L, Irene L, Juliette M-H, Leonhard M, Fanny M-P, Amy SP, Gabriele R, Matthias S, Shimizu H, Eli S, Maurice vS, Alain T, Jorge RT, Pierre V, Anders V, Mary W, Heiko T:
Revised nomenclature and classification of inherited ichthyoses: Results of the First Ichthyosis
Consensus Conference in Sorèze 2009.
J Am Acad Dermatol, in press.

55. Wang G, Ujiie H, Shibaki A, Nishie W, Tateishi Y, Kikuchi K, Li Q, McMillan JR, Morioka H, Sawamura D, Nakamura H, Shimizu H:
Blockade of autoantibody-initiated tissue damage by using recombinant Fab antibody fragments against pathogenic autoantigen.
Am J Pathol, in press.

学会発表

1. Shimizu H, Akiyama M:
The proposal of a clinical classification approach of ichthyosis.
The Second Ichthyosis Consensus Meeting. France, Soreze, 2009/01/23-24

2. Shimizu H:
(Lecture) What' s new in ichthyosis.
Department of Dermatology, Stanford University. California, USA, 2009/03/03

3. Shimizu H:
What' s new in epidermolysis bullosa hereditaria.
The67th Annual Meeting of AAD.
San Francisco, USA, 2009/03/07

4. Fujita Y, Abe R, Inokuma D, Sasaki M, Hoshina D, Nishie W, Natsuga K, McMilan J, Nakamura H, Shimizu H:
Bone marrow transplantation restores deficient epidermal basement membrane protein and improves the clinical phenotype in epidermolysis bullosa model mice.
The 4th Joint Meeting of Japanese Dermatological Association and Australasian College of Dermatologists.
Sapporo, 2009/07/10-12

5. Nomura Y, Abe R, Ota H, Baba K, Hata H, Yamane N, Shimizu H:
A case of disseminated intravascular coagulation associated with Stevens-Johnson syndrome.
The 4th Joint Meeting of Japanese Dermatological Association and Australasian College of Dermatologists.
Sapporo, 2009/07/10-12

6. Ujiie H, Shibaki A, Nishie W, Sawamura D, Wang G, Tateishi Y, Li Q, Nakamura H, Akiyama M, Shimizu H:
Development of an active bullous pemphigoid mouse model.
The 4th Joint Meeting of Japanese Dermatological Association and Australasian College of Dermatologists.
Sapporo, 2009/07/10-12

7. Yanagi T, Akiyama M, Nishihara H, Sakai K, Nishie W, Tanaka S, Shimizu H:
A bcal2.-|- harlequin ichthyosis model mouse shows alveolar collapse as well as severe fetal skin barrier defects.
The 4th Joint Meeting of Japanese Dermatological Association and Australasian College of Dermatologists.
Sapporo, 2009/07/10-12

8. Shimizu H:

Novel animal models for skin diseases: Message from Sapporo.
ADA 129th Annual Meeting. Utah, U.S.A., 2009/08/22

9. McMillan J, Qiao H, Shibaki A, Long H, Wang G, Nishie W, Akiyama M, Shimizu H:
Collagen XVII in hemidesmosomes is involved in keratinocyte-collagen IV adhesion and p38-MAPK-dependent cell migration and signaling.
The 39th Annual Meeting of the European Society for Dermatological Research. Budapest, 2009/09

10. Shimizu H:
Transdifferentiation of bone marrow stem cells into keratinocytes.
EB 2009. Vienna, Austria, 2009/09/07

11. Fujita Y, Abe R, Inokuma D, Nishie W, Nakamura H, Akiyama M, Sawamura D, Shimizu H:
Both hematopoietic and mesenchymal stem cells contribute to the production of basement membrane protein in bone marrow transplantation treatment model mouse.
European Society for Dermatologica Research. Budapest, Hungary, 2009/09/10

12. Long H, Akiyama M, Yanagi T, Shimizu H:
ABCA subfamily member expression in the epidermis is altered by knockdown of ABCA12.
European Society for Dermatologica Research. Budapest, Hungary, 2009/09/10

13. Natsuga K, Nishie W, Akiyama M, Ouchi T, Ishiko A, Hirako Y, Owaribe K, Bruckner-Tuderman L, Shimizu H:
Plectin expression in epidermolysis bullosa simplex (EBS) with muscular dystrophy vs. in EBS with pyloric atresia.
European Society for Dermatologica Research. Budapest, Hungary, 2009/09/10

14. Shimizu H:
(Lecture) Humanization of autoantigens: Lessons from bullous pemphigoid and epidermolysis bullosa.
Klinik und Poliklinik für Dermatologie und Allergologie am Biederstein der TU München Lecture. Munich, Germany, 2009/10/05

15. Shimizu H:
(JSID Symposium) Atopic dermatitis: Its skin barrier function and filaggrin mutations.
The 18th Congress of the European Academy of Dermatology and Venereology. Berlin, Germany, 2009/10/07

16. Shimizu H:
(Plenary Lecture) "Mouse models of human diseases" The 18th Annual Meeting of the European Academy of Dermatology and Venereology. Berlin, Germany, 2009/10/08

17. Shimizu H:
(Lecture) Lessons from novel mice models of inherited and autoimmune blistering diseases.
Department of Dermatology, University of Cologne. Cologne, Germany, 2009/10/12-13

18. Shimizu H:
(Lecture) Mouse models of blistering diseases and ichthyosis.
Department of Dermatology, University of Freiburg. Freiburg, Germany, 2009/10/14

19. Shimizu H:
(Lecture) Bone marrow transplantation therapy for epidermolysis bullosa

mouse model EB meeting. Freiburg, Germany, 2009/10/16

20. Shimizu H:
(Symposium) Harlequin Ichthyosis and Lamellar Ichthyosis: Molecular Diagnosis and Prenatal Diagnosis.
11th World Congress of Pediatric Dermatology. Bangkok, Thailand, 2009/11/17-18

21. Shimizu H:
(Symposium) Lessons from a Novel Mouse Model for Epidermolysis Bullosa.
11th World Congress of Pediatric Dermatology. Bangkok, Thailand, 2009/11/17-18

22. Shimizu H:
(Lecture) Great cases from Hokkaido University, Sapporo.
Institute of Dermatology, Bangkok. Bangkok, Thailand, 2009/11/20

23. Abe R, Yoshioka N, Murata J, Fujita Y, Hoshina D, Mae H, Shimizu H:
Development of a rapid immunochromatographic test for the detection of high-level serum ranulysin in an early stage of SJS and TEN.
The 34th Annual Meeting of the Japanese Society for Investigative Dermatology. Fukuoka, Japan, 2009/12/4-6

24. Fujita Y, Abe R, Inokuma D, Sasaki M, Natsuga K, Nishie W, Nakamura H, Akiyama M, Sawamura D, Shimizu H:
Stem cell transplantation treatment restores deficient type XVII collagen in junctional epidermolysis bullosa model mice.
The 34th Annual Meeting of the Japanese Society for Investigative Dermatology. Fukuoka, Japan, 2009/12/4-6

25. Hoshina D, Abe R, Hata H, Fujita Y, Yoshioka N, Aoyagi T, Shimizu H:
Establishment of a novel experimental model of human angiosarcoma.
The 34th Annual Meeting of the Japanese Society for Investigative Dermatology. Fukuoka, Japan, 2009/12/4-6

26. Long HA, Akiyama M, Yanagi T, Shimizu H:
Expression of ABCA subfamily member in the epidermis is affected by knockout of ABCA12.
The 34th Annual Meeting of the Japanese Society for Investigative Dermatology. Fukuoka, Japan, 2009/12/4-6

27. Natsuga K, Nishie W, Akiyama M, Shinkuma S, Nakamura H, McMillan JR, Nagasaki A, Has C, Ouchi T, Ishiko A, Hirako Y, Owaribe K, Sawamura D, Leena B-T, Shimizu H:
Presence of plectin rodless isoform is a key predictor of phenotypic severity in plectin deficient epidermolysis bullosa simplex.
The 34th Annual Meeting of the Japanese Society for Investigative Dermatology. Fukuoka, Japan, 2009/12/4-6

28. Nemoto-Hasebe I, Akiyama M, Nomura T, Sandilands A, McLean W, Shimizu H:
Novel mutation of FLG, p.Lys4021X in the C-terminal imperfect filaggrin repeat in Japanese atopic dermatitis patients.
The 34th Annual Meeting of the Japanese Society for Investigative Dermatology. Fukuoka, Japan, 2009/12/4-6

29. Inomata K, Aoto T, Binh NT, Okamoto N, Tanimura S, Wakayama T, Iseki S, Hara E, Masunaga T, Shimizu H, Nishimura EK:
Genotoxic Stress Induces Hair Graying Through Melanocyte Stem Cell Differentiation in the niche.

The 34th Annual Meeting of the Japanese Society for Investigative Dermatolgoy. Fukuoka, 2009/12/04-06

30. Niizeki H, Asada H, Amagai M, Oka A, Inoko H, Hashimoto T, Ikeda S, Shimizu H, Kitajima Y, Miyagawa S, Yamamoto S, Aoyama Y, Ichiki Y, Shimizu T, Takahashi H, Kuwana M, Shirakata Y, Hashimoto K, Yamamoto T, Iwatsuki K, Inaoki M, Fujimoto W, Nakamura K, Kishimoto K, Kaneko F, Kunii T, Aiba S, Terui T:
Genetic variation of DSG3 is associated with pemphigus vulgaris among Japanese.
The 34th Annual Meeting of the Japanese Society for Investigative Dermatology. Fukuoka, 2009/12/04-06

H. 知的所有権の出願・登録状況(予定を含む)

該当なし。

I. 引用文献

1. Natsuga K, Nishie W, Akiyama M, Nakamura H, Shinkuma S, McMillan JR, Nagasaki A, Has C, Ouchi T, Ishiko A, Hirako Y, Owaribe K, Sawamura D, Bruckner-Tuderman L, Shimizu H.
Plectin expression patterns determine two distinct subtypes of epidermolysis bullosa simplex.
Hum Mutat, in press

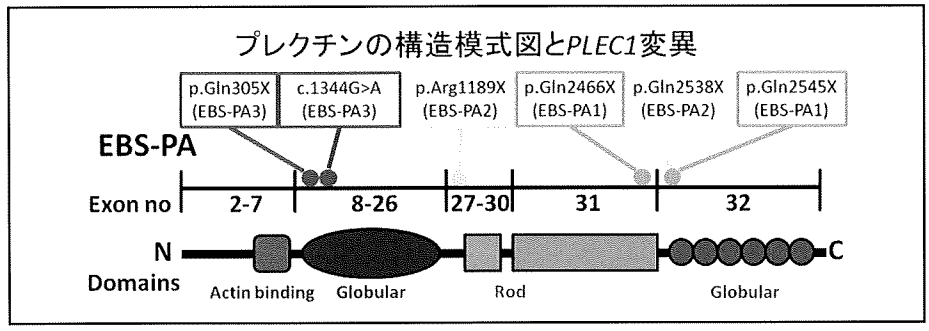
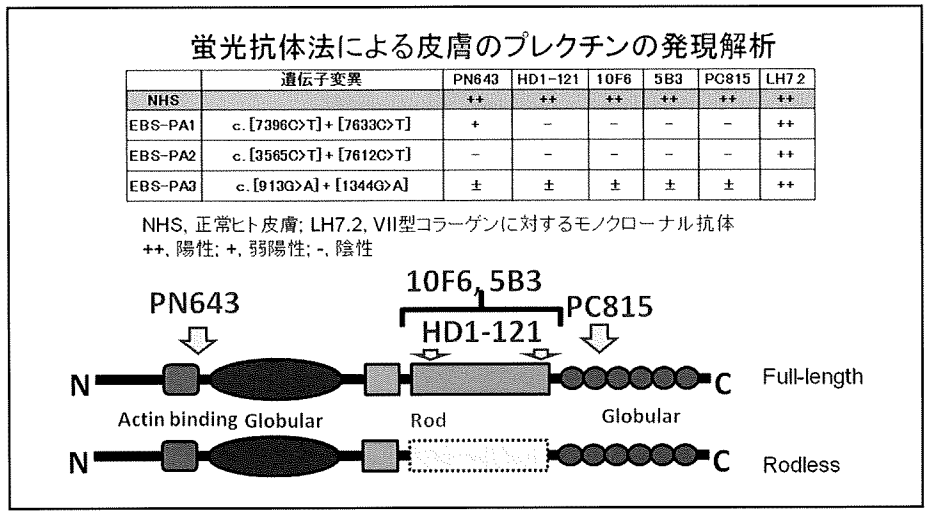


図 1



	遺伝子変異	PN643	HD1-121	10F6	5B3	PC815	LH7.2
NHS		++	++	++	++	++	++
EBS-PA1	c.[7396C>T]+[7633C>T]	+	−	−	−	−	++
EBS-PA2	c.[3565C>T]+[7612C>T]	−	−	−	−	−	++
EBS-PA3	c.[913G>A]+[1344G>A]	±	±	±	±	±	++

図 2

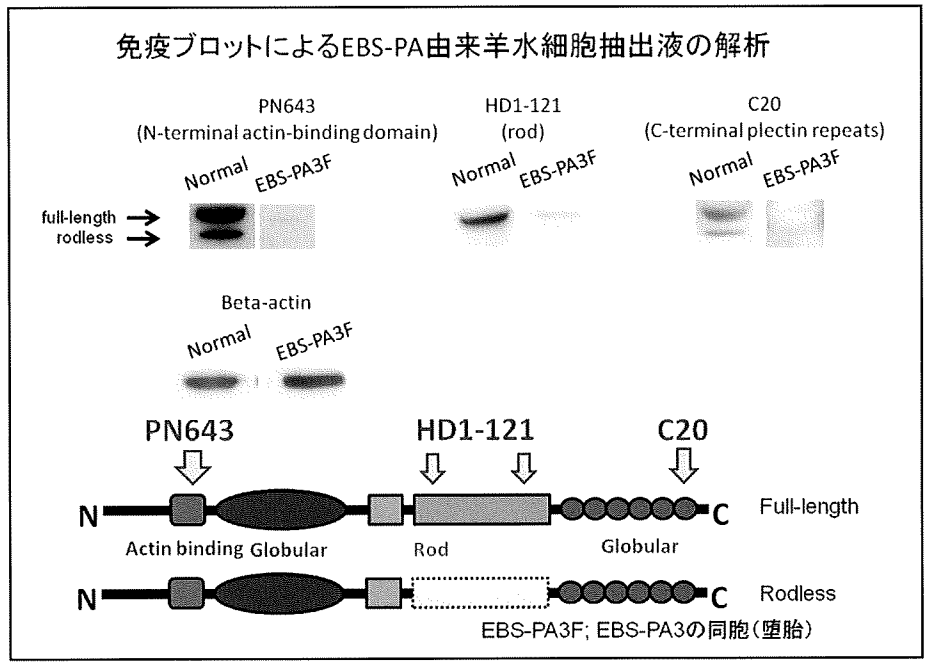


図 3

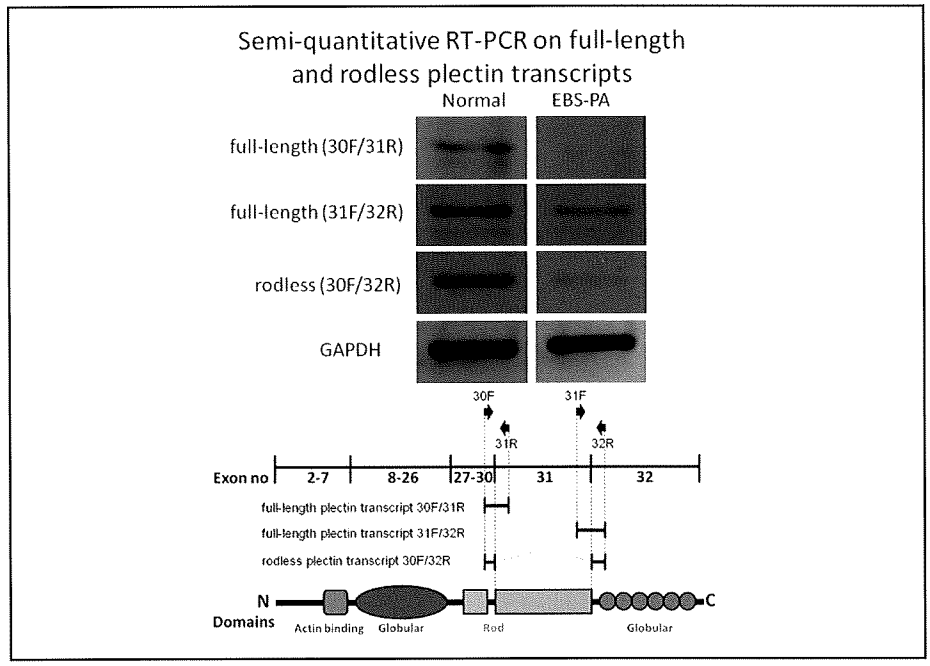
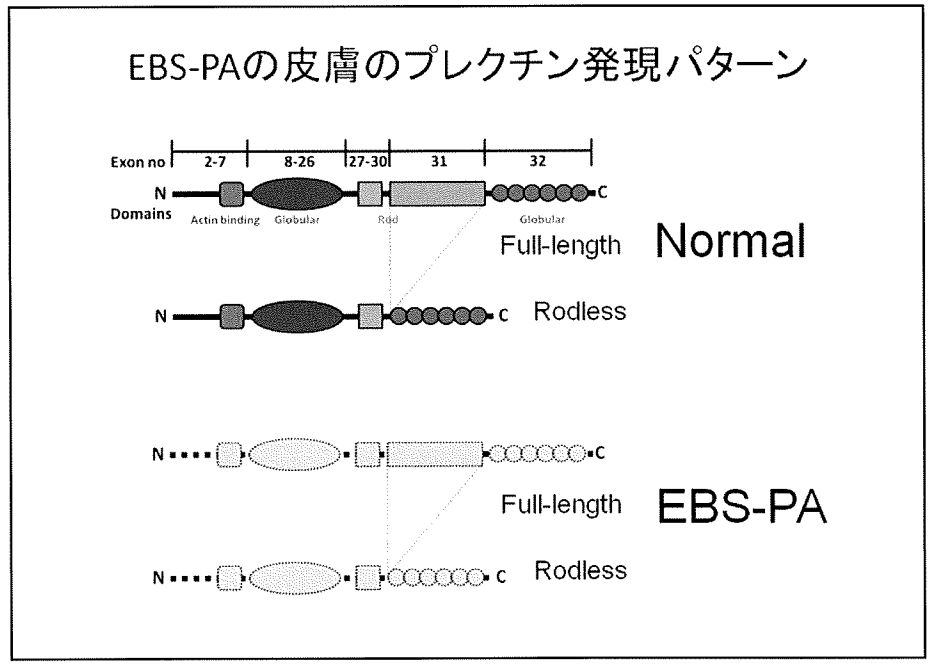

Semi-quantitative RT-PCR on full-length
and rodless plectin transcripts
Normal
EBS-PA
full-length (30F/31R)
full-length (31F/32R)
rodless (30F/32R)
GAPDH
30F
31F
31R
32R
Exon no
2-7
8-26
27-30
31
32
full-length plectin transcript 30F/31R
full-length plectin transcript 31F/32R
rodless plectin transcript 30F/32R
N
C
Domains
Actin binding
Globular
Rod
Globular

図 4


EBS-PAの皮膚のプレクチン発現パターン
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8-26
27-30
31
32
N
C
Domains
Actin binding
Globular
Rod
Globular
Full-length
Normal
Rodless
Full-length
EBS-PA
Rodless

図 5

厚生労働科学研究費補助金（難治性疾患克服研究事業）
分担研究報告書

筋ジストロフィー合併単純型表皮水疱症におけるプレクチンの発現パターン

研究分担者　清水　宏　北海道大学大学院医学研究科皮膚科学分野　教授

研究要旨　プレクチンはプラキンファミリーに属するクロスリンカー蛋白であり、皮膚や筋肉をはじめとした様々な臓器で発現している。プレクチンは、両端のglobular domainにrod domainが挟まれるダンベル状の構造を有している。筋ジストロフィー合併単純型表皮水疱症（EBS-MD）患者のほとんどは、プレクチンのrod domainをコードする*PLEC1*のexon31に遺伝子変異を認める。今回われわれは、プレクチンのN末端、rod domain、C末端に対する抗体を用いて、*PLEC1*の遺伝子変異が確認されたEBS-MD患者の皮膚におけるプレクチンの発現パターンを解析した。また、同時にEBS-MD患者由来の線維芽細胞におけるプレクチンの発現を解析した。EBS-MDでは、全長のプレクチンを発現していないが、rod domainが欠損した蛋白の発現が保たれていた。

共同研究者
夏賀　健　北海道大学皮膚科
西江　渉　北海道大学皮膚科

A．研究目的

プレクチンは、中央のrod domainをN末端とC末端のglobular domainがそれぞれ挟み込む、独特なダンベル様構造を呈する細胞骨格リンカー蛋白であり、皮膚・筋肉・消化管をはじめとした様々な臓器で発現している。プレクチンには全長の蛋白とともに、rod domainが除かれたrodlessプレクチンが選択的スプライシングによって生じることが知られている。

筋ジストロフィー合併型表皮水疱症（EBS-MD）は、プレクチンをコードする*PLEC1*の遺伝子変異によって生じることが知られている。過去の報告では、EBS-MD患者の多くがrod domainをコードするエクソン31に遺伝子変異を有するとされている。本研究の目的はプレクチンの様々な部位に対する抗体を用いることで、EBS-MD患者におけるプレクチンの詳細な発現パターンを解析し、病態解明につなげることである。

B．研究方法

*PLEC1*遺伝子に変異を持つことが証明されているEBS-MDの6症例について患者皮膚を解析した（図1）。プレクチンパターンを蛍光抗体法で解析した。プレクチンのN末端領域、rod領域、C末端領域のそれぞれに対する抗体を用いて、患者皮膚組織のプレクチンの発現パターンを明らかとした。また、EBS-MD患者由来培養線維芽細胞のプレクチン発現パターンについて蛋白レベルとmRNAレベルで解析した。

C．研究結果

EBS-MD皮膚組織の蛍光抗体法の結果、プレクチンのN末端とC末端の発現が減弱しながらも保たれているのに対し、rod domainの発現はほぼ消失していた（図2）。免疫ブロットによる解析では、正常ヒト線維芽細胞抽出液で全長のプレクチンとrodlessプレクチンが発現していたが、EBS-MD由来線維芽細胞抽出液では全長のプレクチンが消失し、rodlessプレクチンのみが発現していた（図3）。mRNAレベルでもEBS-MD由来線維芽細胞では全長のプレクチンをコードする転

写産物が減少しており、逆にrodlessプレクチンの転写産物は正常ヒト線維芽細胞と同じ量を有していた（図４）。

D．考　察

本研究の結果は、ほとんどのEBS-MDの患者で*PLEC1*変異がrod領域をコードするエクソン31に認められるという報告と一致する。*PLEC1*変異がrod領域にあるために、全長のプレクチンはnonsense-mediated mRNA decayによって発現が消失してしまうが、rodlessプレクチンの発現には影響しないと考えられる。

E．結　論

EBS-MDでは、全長のプレクチンは発現していないが、rodlessプレクチンの発現が保たれている（図５）。

F．健康危険情報

特記すべきことなし。

G．研究発表（平成21年度）

論文発表

1．Abe R, Yoshioka N, Murata J, Fujita Y, Shimizu H:
Granulysin as a marker for early diagnosis of the Stevens-Johnson syndrome.
Ann Intern Med 151: 514-515, 2009.

2．Akiyama M, Sakai K, Hayasaka K, Tabata N, Yamada M, Ujiie H, Shibaki A, Shimizu H:
Conradi-Hunermann-Happle syndrome with abnormal lamellar granule contents.
Br J Dermatol 160: 1335-1337, 2009.

3．Amagai M, Ikeda S, Shimizu H, Iizuka H, Hanada K, Aiba S, Kaneko F, Izaki S, Tamaki K, Ikezawa Z, Takigawa M, Seishima M, Tanaka T, Miyachi Y, Katayama I, Horiguchi Y, Miyagawa S, Furukawa F, Iwatsuki K, Hide M, Tokura Y, Furue M, Hashimoto T, Ihn H, Fujiwara S, Nishikawa T, Ogawa H, Kitajima Y, Hashimoto K:
A randomized double-blind trial of intravenous immunoglobulin for pemphigus.
J Am Acad Dermatol 60: 595-603, 2009.

4．Ando S, Abe R, Sasaki M, Murata J, Inokuma D, Shimizu H:
Bone marrow-derived cells are not the origin of the cancer stem cells in ultraviolet-induced skin cancer.
Am J Pathol 174: 595-601, 2009.

5．Aoyagi S, Hata H, Iitani MM, Homma E, Inokuma D, Shimizu H:
Squamous cell carcinoma of the auricle with rhabdoid features.
J Cutan Pathol 36: 919-921, 2009.

6．Aoyagi S, Izumi K, Hata H, Kawasaki H, Shimizu H:
Usefulness of real-time tissue elastography for detecting lymph-node metastases in squamous cell carcinoma.
Clin Exp Dermatol 34: e744-e747, 2009.

7．Asaka T, Akiyama M, Domon T, Nishie W, Natsuga K, Fujita Y, Abe R, Kitagawa Y, Shimizu H:
Type XVII collagen is a key player in tooth enamel formation.
Am J Pathol 174: 91-100, 2009.

8．Asaka T, Akiyama M, Kitagawa Y, Shimizu H:
Higher density of label-retaining cells in gingival epithelium.
J Dermatol Sci 55: 132-134, 2009.

9．Bohgaki T, Atsumi T, Bohgaki M, Furusaki A, Kondo M, Sato-Mat-

sumura KC, Abe R, Kataoka H, Horita T, Yasuda S, Amasaki Y, Nishio M, Sawada K, Shimizu H, Koike T:
Immunological reconstitution after autologous hematopoietic stem cell transplantation in patients with systemic sclerosis: relationship between clinical benefits and intensity of immunosuppression.
J Rheumatol 36: 1240-1248, 2009.

10. Goto-Ohguchi Y, Nishie W, Akiyama M, Tateishi Y, Aoyagi S, Tsuji-Abe Y, Sawamura D, Ishii N, Hashimoto T, Shimizu H:
A severe and refractory case of anti-p200 pemphigoid resulting in multiple skin ulcers and scar formation.
Dermatology 218: 265-271, 2009.
11. Hamasaka A, Abe R, Koyama Y, Yoshioka N, Fujita Y, Hoshina D, Sasaki M, Hirasawa T, Onodera S, Ohshima S, Leng L, Bucala R, Nishihira J, Shimizu T, Shimizu H:
DNA vaccination against macrophage migration inhibitory factor improves atopic dermatitis in murine models.
J Allergy Clin Immunol 124: 90-99, 2009.
12. Hamasaka E, Akiyama M, Hata H, Aoyagi S, Shimizu H:
Melanonychia caused by Stenotrophomonas maltophilia.
Clin Exp Dermatol 34: 242-243, 2009.
13. Honda A, Abe R, Yoshihisa Y, Makino T, Matsunaga K, Nishihira J, Shimizu H, Shimizu T:
Deficient deletion of apoptotic cells by macrophage migration inhibitory factor (MIF) overexpression accelerates photocarcinogenesis.
Carcinogenesis 30: 1597-1605, 2009.
14. Hsu CK, Akiyama M, Nemoto-Hasebe I, Nomura T, Sandilands A, Chao SC, Lee JY, Sheu HM, McLean WH, Shimizu H:
Analysis of Taiwanese ichthyosis vulgaris families further demonstrates differences in FLG mutations between European and Asian populations.
Br J Dermatol 161: 448-451, 2009.
15. Imada K, Dainichi T, Yokomizo A, Tsunoda T, Song YH, Nagasaki A, Sawamura D, Nishie W, Shimizu H, Fukagawa S, Urabe K, Furue M, Hashimoto T, Naito S:
Birt-Hogg-Dube syndrome with clear-cell and oncocytic renal tumour and trichoblastoma associated with a novel FLCN mutation.
Br J Dermatol 160: 1350-1353, 2009.
16. Inokuma D, Aoyagi S, Saito N, Iitani MM, Homma E, Hamasaka K, Shimizu H:
Bowen's disease of the nail matrix presenting as melanonychia: detection of human papillomavirus type 56.
Acta Derm Venereol 89: 638-639, 2009.
17. Inokuma D, Kodama K, Natsuga K, Kasai M, Abe M, Nishie W, Abe R, Hashimoto T, Shimizu H:
Autoantibodies against type XVII collagen C-terminal domain in a patient with bullous pemphigoid associated with psoriasis vulgaris.
Br J Dermatol 160: 451-454, 2009.
18. Inokuma D, Sawamura D, Shibaki A, Abe R, Shimizu H:
Scleroedema adultorum associated with sarcoidosis.
Clin Exp Dermatol 34: e428-429, 2009.
19. Inomata K, Aoto T, Binh NT, Okamoto N, Tanimura S, Wakayama T, Iseki S, Hara E, Masunaga T, Shimizu

H, Nishimura EK:
Genotoxic stress abrogates renewal of melanocyte stem cells by triggering their differentiation.
Cell 137: 1088-1099, 2009.

20. Ito K, Sawamura D, Goto M, Nakamura H, Nishie W, Sakai K, Natsuga K, Shinkuma S, Shibaki A, Uitto J, Denton CP, Nakajima O, Akiyama M, Shimizu H:
Keratinocyte-/fibroblast-targeted rescue of Col7a1-disrupted mice and generation of an exact dystrophic epidermolysis bullosa model using a human COL7A1 mutation.
Am J Pathol 175: 2508-2517, 2009.

21. Kanda M, Natsuga K, Nishie W, Akiyama M, Nagasaki A, Shimizu T, Shimizu H:
Morphological and genetic analysis of steatocystoma multiplex in an Asian family with pachyonychia congenita type 2 harbouring a KRT17 missense mutation.
Br J Dermatol 160: 465-468, 2009.

22. Kawabori M, Kuroda S, Nakayama N, Kenmotsu Y, Shimizu H, Tanino M, Iwasaki Y:
Spontaneous giant aneurysm of the superficial temporal artery: case report.
Neurol Med Chir (Tokyo) 49: 198-201, 2009.

23. Long HA, McMillan JR, Qiao H, Akiyama M, Shimizu H:
Current advances in gene therapy for the treatment of genodermatoses.
Curr Gene Ther 9: 487-494, 2009.

24. McMillan JR, Long HA, Akiyama M, Shimizu H, Kimble RM:
Epidermolysis bullosa (EB) - diagnosis and therapy.
Wound Practice and Research 17: 62-70, 2009.

25. Natsuga K, Shinkuma S, Nishie W, Shimizu H:
Animal models of epidermolysis bullosa.
Dermatol Clin 28: 137-142, 2009.

26. Nemoto-Hasebe I, Akiyama M, Kudo S, Ishiko A, Tanaka A, Arita K, Shimizu H:
Novel mutation p.Gly59Arg in GJB6 encoding connexin 30 underlies palmoplantar keratoderma with pseudoainhum, knuckle pads and hearing loss.
Br J Dermatol 161: 452-455, 2009.

27. Nemoto-Hasebe I, Akiyama M, Nomura T, Sandilands A, McLean WH, Shimizu H:
Clinical severity correlates with impaired barrier in filaggrin-related eczema.
J Invest Dermatol 129: 682-689, 2009.

28. Nemoto-Hasebe I, Akiyama M, Nomura T, Sandilands A, McLean WH, Shimizu H:
FLG mutation p.Lys4021X in the C-terminal imperfect filaggrin repeat in Japanese patients with atopic eczema.
Br J Dermatol 161: 1387-1390, 2009.

29. Nishie W, Sawamura D, Natsuga K, Shinkuma S, Goto M, Shibaki A, Ujiie H, Olasz E, Yancey KB, Shimizu H:
A novel humanized neonatal autoimmune blistering skin disease model induced by maternally transferred antibodies.
J Immunol 183: 4088-4093, 2009.

30. Nomura T, Akiyama M, Sandilands A, Nemoto-Hasebe I, Sakai K, Nagasaki A, Palmer CN, Smith FJ, McLean WH, Shimizu H: