外因性 AD では、内因性 AD と比べ、TEWL が有意に高く、角質水分量も前者のほうが有意に低かった。

③ 末梢血ヘルパーT 細胞局在

内因性 AD では、外因性 AD と比して、IFN-γ陽性 Th1 細胞が増加していた。

④ 金属アレルギー

内因性 AD のほうが、外因性 AD と比べて、コバルトに対する金属アレルギー陽性率が有意に高かった（52.9% vs 10.5%）。

D．考察

内因性 AD は外因性 AD と異なり、フィラグリン遺伝子変異の頻度が低く、バリア機能も正常である。また、IFN-γ陽性 Th1 細胞が増加し、金属アレルギー陽性率が高い。これらの事実は、内因性 AD では皮膚バリア機能は正常であり、タンパク抗原が皮膚に侵入し、AD の病変を形成している可能性は低く、金属などの非タンパク抗原が病態に関与している可能性を強く示唆している。

E．結論

本年度は、外因性 AD と内因性 AD の差異を明らかにすることに成功した。来年度は、外因性 AD とそれに続発する気管支喘息についてさらなる検討を加えていく予定である。

F．健康危険情報

特になし。

G．研究発表

論文投稿中。

H．知的財産の出願・登録状況

特になし。

Ⅳ．研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表（雑誌）

発表者氏名	論文タイトル名	発表雑誌	巻号	ページ	出版年
Umemoto H, **Akiyama M**, Yanagi T, Sakai K, Aoyama Y, Oizumi A, Suga Y, Kitagawa Y, **Shimizu H**	New insight into genotype/phenotype correlations in ABCA12 mutations in harlequin ichthyosis.	**J Dermatol Sci**			in press
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Ⅴ．研究成果の刊行物・別刷

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Letter to the Editor

New insight into genotype/phenotype correlations in *ABCA12* mutations in harlequin ichthyosis

Harlequin ichthyosis (HI) is a severe and often fatal congenital ichthyosis with an autosomal recessive inheritance pattern [1]. The clinical features include thick, plate-like scales with ectropion, eclabium and flattened ears. *ABCA12* mutations underlie HI [2,3] and it was clarified that HI is caused by severe functional defects in the keratinocyte lipid transporter ABCA12 [2]. To date, various *ABCA12* mutations have been reported in HI patients [4]. However, genotype/phenotype correlations in *ABCA12* mutations have been poorly elucidated. In order to obtain clues to understand genotype/phenotype correlations in *ABCA12* mutations, we report two HI patients from two independent Japanese families, who were compound heterozygotes for *ABCA12* mutations.

Patient 1 is the second child of healthy, unrelated Japanese parents. The skin of the baby girl was covered with white, diamond shaped plaques at birth (Fig. 1a). After therapy with oral retinoids and local application of white petrolatum, in a humid incubator, the scales gradually detached and passive and spontaneous mobility of the joints increased. Now at the age of 1 year and 7 months, her general condition is good, although she still has white to grey scales on a background of erythematous skin over her entire body. Patient 2 is the fourth child of healthy, unrelated Japanese parents. Her older brother had a history of congenital ichthyosis and died in early infancy. The skin of the newborn showed serious symptoms with thick, white, diamond shaped plaques, partly bordered by bleeding fissures (Fig. 1c). Although she had therapy with oral retinoids and local application of white petrolatum, in a humid incubator, her clinical symptoms failed to show any apparent improvement and she died when she was 5 months old.

Skin biopsies showed thick stratum corneum in both patients (Fig. 1d–g). In Patient 2, parakeratosis was observed in the epidermis and a sparse inflammatory cell infiltration was seen in the superficial dermis (Fig. 1e inset). Electron microscopy (Hitachi, Tokyo, Japan) revealed a large number of abnormal, variously sized lipid droplets that accumulated in the cornified cells of both patients' epidermis.

Mutational analysis of *ABCA12* was performed in both patients and their families. Each genomic DNA sample was subjected to PCR amplification, followed by direct automated sequencing. Oligonucleotide primers and PCR conditions used for amplification of all exons 1–53 of *ABCA12* were originally derived from the report by Lefèvre et al. [5] and were partially modified for the present study. The entire coding region including the exon/intron boundaries for both forward and reverse strands from the patients, their parents and 50 healthy Japanese controls were also sequenced. Both patients had the same paternal novel nonsense mutation p.Arg1515X (Fig. 1h) which leads to truncation of the first ATP-binding cassette within ABCA12 likely resulting in ABCA12 loss of function (Fig. 2a). On the other allele, Patient 1 had a maternal recurrent splice acceptor site mutation c.3295-2A>G (Fig. 1h). This splice site mutation was reported in an unrelated Japanese family with HI and was shown to lead to comparable amounts of 2 splice pattern variants [2]. The first mutant transcript would result in a 3 amino acids deletion (1099_1101delYMK). These 3 amino acids are located in the first transmembrane domain and are highly conserved (Fig. 2b). The second mutant transcript lost a 170-bp sequence from exon 24, which led to a frameshift. Expression of a small amount of ABCA12 protein, although mutated, was detected in the granular layer keratinocytes of the patient's epidermis and cultured keratinocytes by immunofluorescent staining [2]. Thus, it is possible that Patient 1 expresses some mutated ABCA12 protein with a partial function. This might be the reason why Patient 1 survived beyond the perinatal and neonatal period and is still alive although this might also be in part due to the prompt oral retinoid treatment.

Patient 2 carried a maternal missense mutation p.Gly1179Arg on the other locus (Fig. 1h). To confirm the presence of the mutation p.Gly1179Arg in Patient 2, we performed restriction enzyme digestion analysis using *BclI* (NEW ENGLAND BioLabs). Restriction enzyme digestion of PCR products was carried out according to the manufacture's protocols. The 255-bp PCR products from wild type alleles were not digested by *BclI*, although the PCR products from the allele with the mutation p.Gly1179Arg were digested into 173- and 82-bp fragments. The father's PCR product after *BclI* digestion showed a single 255-bp band, which indicated he had only normal alleles. In contrast, the PCR product after *BclI* digestion from the mother of Patient 2 showed 255-, 173- and 82-bp bands, which indicated that she was heterozygous for the p.Gly1179Arg missense mutation (supplementary Fig. S1). This mutation was reported in a Laotian family [6]. The glycine 1179 is a highly conserved amino acid residue (Fig. 2b) located in the first transmembrane ABCA12 domain (Fig. 2a), and this mutation substitutes an uncharged polar glycine residue for a positively charged arginine residue. The presence of these mutations was excluded in 100 alleles of 50 normal unrelated Japanese individuals.

Determinants of genotype/phenotype correlations resulting from *ABCA12* mutations, typically demonstrate that homozygotes or compound heterozygotes with truncation *ABCA12* mutations lead to an HI phenotype. Only a few exceptional cases have been reported such as the present case. The mutation p.Gly1179Arg might result in major loss of ABCA12 function and/or structure, leading to the severe phenotype in Patient 2.

Recently, long-term survival of patients with HI has been more frequently observed and documented [7,8]. The clinical symptoms of Patient 1 showed a remarkable improvement during infancy. In contrast, the symptoms of Patient 2 did not improve, and she died at the age of 5 months. The marked difference in the clinical severity of the two patients indicated that the p.Gly1179Arg has far bigger deleterious functional effects than c.3295-2A>G. The present study clearly demonstrates that some missense *ABCA12*

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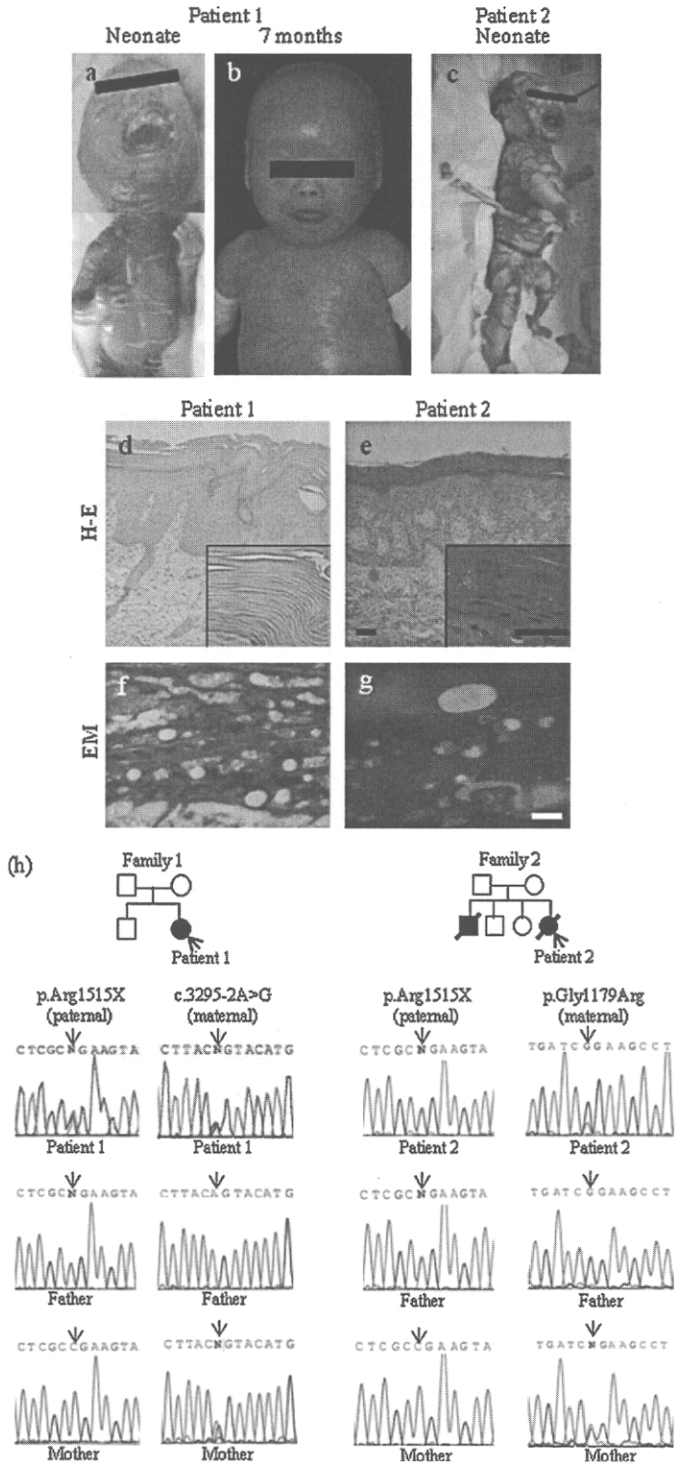

Fig. 1. (a–c) Clinical features of HI patients. Patient 1 showed the typical clinical phenotype of HI during the neonatal period, including the face and trunk (a). Her clinical symptoms remarkably improved at 7 months of age (b). Patient 2 showed more serious symptoms with thick plate-like scales and skin fissures in the neonatal period (c) and lived until the age of 5 months. (d–g) Histological features of the skin lesions of HI patients. Skin biopsies showed thick stratum corneum in both patients. Bars, 50 μm (d and e). In Patient 2, parakeratosis were observed (e, inset). By electron microscopy, abnormal variously sized lipid droplets had accumulated in the cornified cells of both patients' epidermis. Bars, 200 nm (f and g). (h) Families with HI and *ABCA12* mutations. Patient 1 was a compound heterozygote for two *ABCA12* mutations, a novel nonsense mutation p.Arg1515X and a recurrent splice site mutation c.3295-2A>G, and both her parents were heterozygous carriers. Patient 2 harboured two *ABCA12* mutations, p.Arg1515X and p.Gly1179Arg, and both her parents were heterozygous carriers of these defects.

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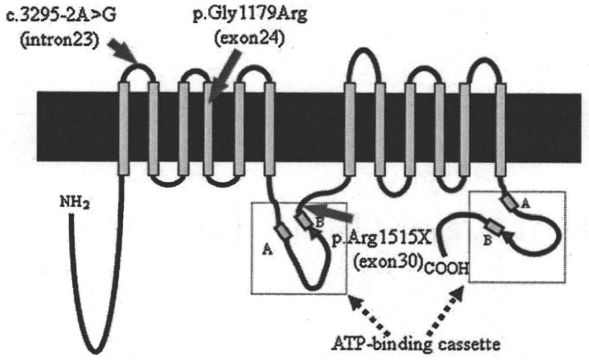

c.3295-2A>G: 1099_1101delYMK

Homo sapiens	1089	VYEKDLRLHEYMKMMGVNSCSHF 1111
Rattus norvegius		VYEKDLRLHEYMKMMGVNSCSHF
Mus musculus		VYEKDLRLHEYMKMMGVNSCSHF
Gallus gallus		VQEKDLRLYEYMKMMGVNASSHF
Danio rerio		VHERELRLHEYMKMMGVNPISHF

p.Gly1179Arg

Homo sapiens	1165	ISVFFNNTNIAALIGSLIYIIAFFPFIVL 1193
Rattus norvegius		ISVFFNNTNIAALIGSLIYVIAFFPFIVL
Mus musculus		ISVFFNNTNIAALIGSLIYVIAFFPFIVL
Gallus gallus		ISVFFNNTNIAALVGSLVYILTFFPFIVL
Danio rerio		VSSFFDKTNIAGLSGSLIYVISFFPFIVL

Fig. 2. (a) Structure of ABCA12 protein and the three mutations in present HI families. Dark blue area, cell membrane; bottom of dark-blue area, cytoplasmic surface. Note the mutation shared between the two patients is a truncation mutation in the first ATP-binding cassette (p.Arg1515X). The other mutation in Patient 2 is just a missense mutation in the first cluster of transmembrane domains (p.Gly1179Arg). (b) ABCA12 amino acid sequence alignment shows the level of conservation in diverse species of the amino acids, 1099_1101delYMK and p.Gly1179Arg (red characters).

mutations within highly conserved transmembrane regions are able to cause drastic changes in protein structure and function, leading to severe phenotypes, similar to truncation mutation patients. Further accumulation of similar cases is needed to confirm genotype/phenotype correlation in *ABCA12* mutations, especially in studies involving missense mutations underlying HI.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jdermsci.2010.11.010.

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Dermatologic Clinics

Pathogenesis of Bullous Pemphigoid

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Synopsis

Bullous pemphigoid, the most common autoimmune blistering diesease, is induced by autoantibodies against type XVII collagen. Passive transfer of IgG or IgE antibodies against type XVII collagen into animals has revealed not only the pathogenicity of these antibodies, but also the subsequent immune responses, including complement activation, mast cell degranulation, and infiltration of neutrophils and/or eosinophils. In vitro studies on ectodomain shedding of type XVII collagen have also provided basic knowledge on the development of bullous pemphigoid. The pathogenic role of autoreactive $CD4^+$ T lymphocytes in the development of the pathogenic autoantibodies to type XVII collagen should also be noted.

Introduction

Bullous pemphigoid (BP), the most common autoimmune blistering disorder, is induced by autoantibodies against the components of the skin basement membrane zone (BMZ).[1, 2] Clinically, tense blisters and erosions with itchy urticarial plaques and erythema develop on the whole body (Fig. 1a). Histological examination of lesional skin reveals subepidermal blisters with inflammatory infiltration consisting of eosinophils and lymphocytes (Fig. 1b). Direct immunofluorescence (IF) shows linear deposition of IgG and complement C3 at the dermal-epidermal junction (DEJ) (Fig. 1c). In addition, indirect IF using the patient's sera demonstrates linear deposition of IgG at the DEJ of normal human skin, and the autoantibodies usually deposit on the roof side of the artificial split-skin blister induced by 1M sodium chloride (Fig. 1d). Immunoblotting reveals that the autoantibodies usually react with 180-kDa or 230-kDa proteins in epidermal extractions of normal human skin as candidate autoantigens. The 230-kDa protein, called BP230 or BPAG1, is a plakin family protein that was originally identified as the major antigen for BP.[3, 4] BP230 is a cytoplasmic component of hemidesmosomes that enhances the linkage of keratin intermediate filaments to hemidesmosomes.[5] Although several studies have indicated that BP230 is pathogenic,[6, 7] it remains unclear whether the autoantibodies against BP230 are pathogenic. At the

moment, the 180-kDa protein is considered to be the main pathogenic antigen in BP.

The major pathogenic antigen in BP: type XVII collagen (COL17)

Autoantibodies against the hemidesmosomal antigen of type XVII collagen (COL17, also called BP180 or BPAG2), a 180-kDa protein, are thought to induce the inflammatory process, resulting in dermal-epidermal separation. COL17 is a type II transmembrane protein that spans the lamina lucida and projects into the lamina densa of the BMZ (Fig. 2a).[8-10] COL17 has 15 collagenous domains in the extracellular domain (Fig. 2b).[11] The noncollagenous 16A (NC16A) domain in the juxtamembranous extracellular part is considered to have the major pathogenic epitope for BP (Fig. 2b).[12, 13] Interestingly, the extracellular part of COL17 is constitutively shed from the cell surface within the NC16A domain.[14]

Epitope mapping using several fragments of COL17 and ELISA analysis has elucidated that sera from most BP patients recognize NC16A.[12, 15] The titer of anti-COL17 NC16A antibodies has been shown to correlate with the disease severity of BP.[16] Autoantibodies against COL17 other than its NC16A domain are also detected in BP sera. About half of the BP sera recognizes the C-terminal regions of the extracellular domain of COL17, and the presence of autoantibodies against both NC16A and the

C-terminal portions of COL17 seems to be associated with the clinical involvement of mucosal lesions in BP patients.[17] Epitope spreading has been suggested as a mechanism for the generation of autoantibodies against various parts of COL17 in BP patients.[18] Intramolecular epitope spreading within COL17 has been demonstrated in an animal model developed by grafting human COL17-expressing transgenic (Tg) mice skin onto wild-type mice.[19] The pathogenic role of autoantibodies against COL17 other than its NC16A domain has not been fully elucidated.

Recently, precise cleavage sites within the NC16A domain of COL17 have been reported.[20] Importantly, cleavage of collagen XVII was shown to generate neoepitopes around amino-terminal cleavage sites on the shed ectodomain. It is well known that autoantibodies from patients with BP[21] as well as from patients with linear IgA bullous dermatosis[22] preferentially recognize the shed ectodomain of COL17, one explanation for which could be that these autoantibodies recognize shedding-generating neoepitopes.[20]

The pathogenicity of anti-COL17 IgG antibodies from BP patients (BP-IgG) has been shown in vitro. BP-IgG against recombinant COL17 NC16A caused dermal-epidermal separation in cryosections of human skin when the skin was incubated with leukocytes from healthy volunteers.[23] In addition, polyclonal rabbit

antibodies that target the shedding-generating neoepitopes also demonstrated the potential to induce dermal-epidermal separation in human skin cryosections.[20] Furthermore, antibodies reacted with the nonblistering regions at the periphery of blister in BP patients, suggesting the presence of neoepitopes in the early stage of BP that are likely to be involved in the pathogenesis of BP.[20]

While some previous studies mentioned the pathogenic role of complement activation in BP, Iwata et al. have reported that only BP-IgG is able to deplete the expression of COL17 in cultured normal human keratinocytes and reduce the attachment of cells from the dish in a complement-independent manner.[24] This suggests that BP-IgG could reduce the content of hemidesmosomal COL17, resulting in weakness of the adhesion of hemidesmosomes to the lamina lucida.

In vivo studies on BP

The pathogenic role of antibodies against COL17 has been shown in a passive transfer mouse model using rabbit IgG antibodies against the murine homolog of human COL17 NC16A (murine COL17 NC14A).[25] The injected neonatal mice demonstrate skin fragility associated with the linear deposition of rabbit IgG and mouse C3 at the DEJ of their skin, and subepidermal separation with inflammatory cell infiltration; these

correspond to the clinical, histological and immunopathological features of BP.[25] Using this experimental BP model, Liu et al. revealed that subepidermal blister formation in their neonatal mouse model depends on complement activation,[26] mast cell degranulation,[27] and neutrophil infiltration.[28] They also showed that the degradation of COL17 in that model depends on neutrophil elastase secreted by infiltrating neutrophils.[29]

Passive transfer of BP-IgG fails to induce a BP-like phenotype in mice, which is explained by the low similarity of the NC16A amino acid sequence between humans and mice. To further investigate the pathogenic roles of anti-human COL17 antibodies, a Tg mouse expressing human COL17 (hCOL17) cDNA driven under the control of a keratin 14 promotor was generated.[30] Olasz et al. demonstrated that wild-type mice grafted with hCOL17 Tg skin produce a high level of anti-hCOL17 IgG and lose the Tg skin grafts with the deposition of IgG and C3 at the DEJ and with a neutrophil infiltration, resulting in the microscopic subepidermal blisters that are observed in BP.[30] These findings show that the anti-hCOL17 IgG induced by Tg skin grafting is pathogenic against Tg skin that expresses hCOL17 antigens. They also demonstrated that major histocompatibility (MHC) class $II^{-/-}$ mice grafted with Tg skin develop neither anti-hCOL17 IgG nor graft loss, indicating that MHC II and $CD4^+$ T cell