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

遺伝性汎発性色素異常症の原因遺伝子同定

研究分担者　山下利春　札幌医科大学医学部皮膚科教授

研究要旨　遺伝性対側性色素異常症（DSH）は遺伝子診断により確定診断を行っているが、DSHの亜型と考えられていた遺伝性汎発性色素異常症（DUH）の原因遺伝子はいまだ不明である。DUHと考えられる皮疹を持つ患者の家系を集積し、次世代シークエンサーを用いた手法で本疾患の原因遺伝子の同定を目指す。

A．研究目的

遺伝性対側性色素異常症（DSH）は遺伝子診断により確定診断を行っているが、DSHの亜型と考えられていた遺伝性汎発性色素異常症（DUH）の原因遺伝子はいまだ不明である。DUHと考えられる皮疹を持つ患者の家系を集積し、次世代シークエンサーを用いた手法で本疾患の原因遺伝子の同定を目指す。

B．研究方法

臨床的にDUHと考えられる患者が見つかった際に、臨床症状の記録および家系調査を行う。孤発例ではなく、少なくとも患者の前後1世代ずつに患者が存在する家族例である症例が見つかった際に名古屋大学皮膚科学教室と連携し、患者および家族に文書を用いて説明をし、同意を得られれば、採血を行い、ゲノムDNAを抽出する。SureSelectエキソームキャプチャーキットを用いて、全エクソン領域を選択し、ABI SOLiDで全エクソン領域の塩基配列決定を行う。そのデータから、変異を持つ遺伝子を候補遺伝子としてあげる。家系内の患者および非罹患者についてそれらの変異を調べて、候補遺伝子を絞り込んでいく。皮膚での発現や機能によっても更に候補遺伝子を絞り込み、最終的に原因遺伝子を同定する。

（倫理面への配慮）

本研究は名古屋大学医学部生命倫理委員会の承認申請中であり、承認が得られたのちに札幌医科大学医学部の生命倫理委員会の承認申請を行う予定である。承認後に研究を行うにあたって、患者の人権および利益の

保護を遵守するために以下の対策を講じることとする。

(1) 患者本人に疾患遺伝子解析の内容と必要性を十分に説明し、個人情報を秘匿するための対応についても説明し、予め作成しておいた書面による同意を得てから検体を採取する。なお、未成年の患者の場合は保護者に同様の説明をした後に保護者に署名をしてもらう。

(2) 採取した検体は個人IDで管理し、個人IDで連結できる患者の情報は鍵をかけた書庫で管理し、個人情報の流出を出来るだけ防ぐ手段をとる。

C．研究結果

現在、協力していただける家系を探索中であり、まだ遺伝子解析は行っていない。

F．健康危険情報

特になし。

G．研究発表

1．論文発表

1) Uhara H, Yamazaki N, Takata M, Inoue Y, Sakakibara A, Nakamura Y, Suehiro K, Yamamoto A, Kamo R, Mochida K, **Yamashita T**, Takenouchi T, Takenaka H, Yoshikawa S, Takahashi A, Uehara J, Kawai M, Iwata H, Kadono T, Kai Y, Watanabe S, Murata S, Ikeda T, Fukamizu H, Tanaka T, Hatta N, Saida T: Applicability of radiocolloids, blue dye and fluorescent indocyanine green to sentinel node biopsy in melanoma.
J Dermatol (in press)

2) Yoneta A, Horimoto K, Nakahashi K, Mori S, Maeda K, **Yamashita T**: A case of cystic basal cell carcinoma which shows a homogenous blue/black area under dermatoscopy.
J Skin Cancer 2011: 450472, 2011.

3) T Hida, H Sohma, Y Kokai, A Kawakami, K Hirosaki, M Okura, N Tosa, **T Yamashita**, K Jimbow. Rab7 is a critical mediator in vesicular transport of tyrosinase-related protein 1 in melanocytes. **Journal of Dermatology** 38: 432-441, 2011.

4) Hida T, Yoneta A, Nishizaka T, Ohmura T, Suzuki Y, Kameshima H, **Yamashita T**. Pigmented mammary Paget's disease mimicking melanoma: Report of three cases.
Eur J Dermatol 1167-1122, 2011.

2．学会発表

1) Okura M, Hagiwara K, Hida T, Yoneta A, Yanagisawa K, Horio Y, **Yamashita T**: Effects of a low-molecular-weight polyphenol(oligonol) on the growth and melanogenesis of primary melanocytes and melanoma cells.
International Pigment Cell Conference. Sep20-24, 2011 . Bordeaux, France.

2) Yoneta A, Tamura Y, Nohara S, Ito A, Honda H, Wakamatsu K, Ito A, **Yamashita T**, Jimbow K: Development and evaluation of antitumor effect of novel NPrCAP-magnetite nanoparticles for chemothermo-immunotherapy in malignant melanoma.
International Pigment Cell Conference. Sep20-24, 2011 . Bordeaux, France.

3) Ishii-Osai Y, **Yamashita T**, Okura M, Tamura Y, Sato N, Ito A, Honda H, Wakamatsu K, Ito S, Jimbow K: N-Propionyl-4-S-Cysteminylphenol generates reactive oxygen species and mediates apoptosis in pigmented melanoma cells.
International Pigment Cell Conference. Sep20-24, 2011 . Bordeaux, France.

H．知的所有権の取得状況

1. 特許取得
特になし。

2. 実用新案登録
特になし。

3. その他
特になし。

IV. 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	発表誌名	巻号	ページ	出版年
Kono M, Akiyama M, Suganuma M, Sanchez-Valle A, Tomita Y.	Dyschromatosis symmetrica hereditaria by ADAR1 mutations and viral encephalitis: a hidden link?	**Int J Dermatol**			in press
Kono M, Akiyama M, Kondo T, Suzuki T, Suganuma M, Wataya-Kaneda M, Lam J, Shibaki A, Tomita Y.	Four novel *ADAR1* gene mutations in patients with dyschromatosis symmetrica hereditaria.	**J Dermatol**			in press
Kono M, Suganuma M, Ito Y, Ujiie H, Morimoto K, Akiyama M.	Novel *ADAR1* mutations including single amino acid deletion in the deaminase domain underly dyschromatosis symmetrica hereditaria in Japanese families	**Int J Dermatol**			in press
Kono M, Kondo T, Ito S, Suzuki T, Wakamatsu K, Ito S, Tomita Y.	Genotype analysis in a patient with oculocutaneous albinism 1 minimal pigment type.	**Br J Dermatol**	166	896-898	2012
Sugiura K, Takeichi T, **Kono M**, Ogawa Y, Shimoyama Y, Muro Y, **Akiyama M**.	A novel IL36RN/IL1F5 homozygous nonsense mutation, p.Arg10X, in a Japanese patient with adult-onset generalized pustular psoriasis.	**Br J Dermatol**			in press

Sugiura K, Takeichi T, **Kono M**, Ito Y, Ogawa Y, Muro Y, **Akiyama M**.	Severe Chilblain Lupus Is Associated with Heterozygous Missense Mutations of Catalytic Amino Acids or their Adjacent Mutations in the Exonuclease Domains of 3'-Repair Exonuclease 1.	**J Invest Dermatol**			in press
Mori M, Sugiura M, **Kono M**, Matsumoto T, Sawada M, Yokota K, Yasue S, Shibata S, Sakakibara A, Nakamura S, Tomita Y, **Akiyama M**.	Clinico-pathologic analysis of 66 Japanese thin melanomas with metastasis of sentinel or regional lymph node.	**J Cutan Pathol**			in press
Hane H, Kenji Yokota K, **Kono M**, Muro Y, **Akiyama M**	Extraordinarily large, giant spider angioma in an alcoholic cirrhotic patient	**Int J Dermatol**			in press
Natsuga K, Shinkuma S, Kanda M, Suzuki Y, Chosa N, Narita Y, Setoyama M, Nishie W, **Akiyama M**, Shimizu H.	Possible modifier effects of keratin 17 gene mutation on keratitis-ichthyosis-deafness syndrome.	**Br J Dermatol**			in press
Yasuda K, Sugiura K, Ishikawa R, Kihira M, Negishi Y, Iwayama H, Ito K, Kimura H, Kosugi I, **Akiyama M**.	Perinatal cytomegalovirus-associated bullae in an immunocompetent infant.	**Arch Dermatol**	148	770-772	2012

Muro Y, Sugiura K, Hoshino K, **Akiyama M**.	Disappearance of anti-MDA-5 autoantibodies in clinically amyopathic dermatomyositis/interstitial lung disease during disease remission.	**Rheumatology**	51	800-804	2012
Karasawa T, Matsumoto T, **Akiyama M**.	Metastatic skin lesions of multiple myeloma presenting as two extraordinarily large subcutaneous tumors.	**Int J Dermatol**			in press
Fukuda S, Hamada T, Ishii N, Sakaguchi S, Sakai K, **Akiyama M**, Shimizu H, Masuda K, Izu K, Teye K, Tsuruta D, Karashima T, Nakama T, Yasumoto S, Hashimoto T.	Novel ATP-binding cassette, subfamily A, member 12 (ABCA12) mutations associated with congenital ichthyosiform erythroderma.	**Br J Dermatol**	166	218-221	2012
Mizuno O, Yanagi T, Baba K, Yamane N, Inokuma D, Ito K, **Akiyama M**, Shimizu H.	Sweet's syndrome presenting with vegetative nodules on the hands: relationship to neutrophilic dermatosis of the dorsal hands.	**Int J Dermatol**			in press
Izumi K, Yanagi T, **Akiyama M**, Moriuchi R, Arita K, Shimizu H.	Intractable erythematous plaques on the hands: palmoplantar eosinophilic pustular folliculitis.	**Int J Dermtol**			in press

Sasaki K, **Akiyama M**, Yanagi T, Sakai K, Miyamura Y, Sato M, Shimizu H.	CYP4F22 is highly expressed at the site and onset of keratinization during human skin development.	**J Dermatol Sci**			in press
Uhara H, Yamazaki N, Takata M, Inoue Y, Sakakibara A, Nakamura Y, Suehiro K, Yamamoto A, Kamo R, Mochida K, **Yamashita T**, Takenouchi T, Takenaka H, Yoshikawa S, Takahashi A, Uehara J, Kawai M, Iwata H, Kadono T, Kai Y, Watanabe S, Murata S, Ikeda T, Fukamizu H, Tanaka T, Hatta N, Saida T	Applicability of radiocolloids, blue dye and fluorescent indocyanine green to sentinel node biopsy in melanoma.	**J Dermatol**	39	336-338	2012
Washio K, Oka M, Ohno K, Shimizu H, Kawano S, Kunisada M, **Nishigori C**.	Case of recurrent Sweet's syndrome in a patient with relapsing polychondritis and myelodysplastic syndrome.	**J Dermatol**			in press
Fukunaga A, Shimizu H, Tanaka M, Kikuzawa A, Tsujimoto M, Sekimukai A, Yamashita J, Horikawa T, **Nishigori C**.	Limited Influence of Aspirin Intake on Mast Cell Activation in Patients with Food-dependent Exercise-induced Anaphylaxis: Comparison Using Skin Prick and Histamine Release Tests.	**Acta Derm Venereol**			in press

Terai M, Oka M, Tsujimoto M, Kuni sada M, Tada S, B ito T, **Nishigori C**.	Recalcitrant pruritic urticarial papules and plaques of pregnancy with a prolonged course after delivery.	**Eur J Dermat ol**	22	136-137	2012
Hatakeyama M, Fukunaga A, Shimizu H, Oka M, Horikawa T, **Nishigori C**.	Drug fever due to S-carboxymethyl-L-cys tein: Demonstration of a causative agent with patch tests.	**J Dermatol**	39	555-556	2012
Taguchi K, Fukunaga A, Okuno T, **Nishigori C**.	Successful treatment with adapalene of cetuximab-induced acneiform eruptions.	**J Dermatol**			in press
Fujiwara S, Nagai H, Oniki S, Yoshimoto T, **Nishigori C**.	Interleukin (IL)-17 versus IL-27: opposite effects on tumor necrosis factor-α-mediated chemokine production in human keratinocytes.	**Exp Dermatol**	21	70-72	2012
Kaibuchi-Noda K, Yokota K, Matsumoto T, Sawada M, Sakakibara A, **Kono M**, Tomita Y, Watanabe D, Fukumoto H, Katano H, **Akiyama M**.	Detection of Merkel cell polyomavirus in cutaneous squamous cell carcinoma prior to occurrence of Merkel cell carcinoma.	**J Am Acad Dermatol**	65	e152-4	2011
Takeichi T, Sugiura K, Muro Y, **Akiyama M**.	LEDGF/DFS70 activates the MK2/IL6/STAT3 pathway in HaCaT.	**J Dermatol Sci**	63	203-205	2011

Li Q, Frank M, **Akiyama M**, Shimizu H, Ho S-Y, Thisse C, Thisse B, Sprecher E, Uitto J.	Abca12-Mediated Lipid Transport and Snap29-Dependent Trafficking of Lamellar Granules are Critical for Epidermal Morphogenesis in Zebrafish Disease Model of Ichthyosis.	**Dis Model Mech**	4	777-785	2011
Natsuga K, **Akiyama M**, Shimizu H.	Malignant skin tumours in inherited ichthyosis patients.	**Br J Dermatol**	165	263-268	2011
Yanagi T, **Akiyama M**, Nishihara H, Miyamura Y, Sakai K, Tanaka S, Shimizu H.	AKT plays an anti-apoptotic role in ABCA12-deficient keratinocytes.	**J Invest Dermatol**	131	1942-1945	2011
Natsuga K, Nishie W, Smith BJ, Shinkuma S, Smith TA, Parry DAD, Oiso N, Kawada A, Yoneda K, **Akiyama M**, Shimizu H.	Consequences of two different amino acid substitutions at the same codon in *KRT14* indicate definitive roles of structural distortion in epidermolysis bullosa simplex pathogenesis.	**J Invest Dermatol**	131	1869-1876	2011
Nakajima K, Uchida Y, **Akiyama M**, Morita Y, Shimizu H, Sano S.	Altered lipid profiles in the stratum corneum of Sjögren-Larsson syndrome.	**J Dermatol Sci**	63	64-66	2011
Akiyama M.	The roles of ABCA12 in keratinocyte differentiation and lipid barrier formation in the epidermis.	**Dermato-Endocrinology**	3	107-112	2011(Review article)
Akiyama M.	Updated molecular genetics and pathogenesis of ichthyoses.	**Nagoya J Med Sci**	73	79-90	2011(Review article)

Suga H, Tsunemi Y, Sugaya M, Shinkuma S, **Akiyama M**, Shimizu H, Sato S.	Hair shaft abnormalities in localized autosomal recessive hypotrichosis 2 and a review of other non-syndromic human alopecias.	**Acta Dermatovenereol**	91	486-488	2011
Kikuchi K, Arita K, Tateishi Y, Onozawa M, **Akiyama M**, Shimizu H.	Recurrence of hydroxyurea-induced leg ulcer after discontinuation of treatment.	**Acta Dermatovenereol**	91	373-374	2011
Nakamura H, Natsuga K, Nishie W, McMillan JR, Nakamura H, Sawamura D, **Akiyama M**, Shimizu H.	DNA-based prenatal diagnosis of plectin-deficient epidermolysis bullosa simplex associated with pyloric atresia.	**Int J Dermatol**	50	439-442	2011
Furue M, Yamazaki S, Jimbow K, Tsuchida T, Amagai M, Tanaka T, Matsunaga K, Muto M, Morita E, **Akiyama M**, Soma Y, Terui T, Manabe M.	Prevalence of dermatological disorders in Japan: A nationwide, cross-sectional, seasonal, multicenter, hospital-based study.	**J Dermatol**	38	353-363	2011
Osawa R, **Akiyama M**, Izumi K, Ujiie H, Sakai K, Nemoto-Hasebe I, Yanagi T, Koizumi H, Shimizu H.	Extremely severe palmoplantar hyperkeratosis in a generalized epidermolytic hyperkeratosis patient with a keratin 1 gene mutation.	**J Am Acad Dermatol**	64	991-993	2011
Yoneta A, Horimoto K, Nakahashi K, Mori S, Maeda K, **Yamashita T**	A case of cystic basal cell carcinoma which shows a homogenous blue/black area under dermatoscopy.	**J Skin Cancer**	2011	450472	2011

T Hida, H Sohma, Y Kokai, A Kawakami, K Hirosaki, M Okura, N Tosa, T Yamashita, K Jimbow.	Rab7 is a critical mediator in vesicular transport of tyrosinase-related protein 1 in melanocytes.	**Journal of Dermatology**	38	432-441	2011
Hida T, Yoneta A, Nishizaka T, Ohmura T, Suzuki Y, Kameshima H, **Yamashita T**.	Pigmented mammary Paget's disease mimicking melanoma: Report of three cases.	**Eur J Dermatol**	22(1)	121-124	2012
Chiyomaru K, Nagano T, **Nishigori C**.	Polymorphisms of glutathione s-transferase in skin cancers in a Japanese population.	**Kobe J Med Sci**	57	E11-16	2011
Masuoka E, Bito T, Shimizu H, **Nishigori C**.	Dysfunction of melanocytes in photoleukomelanoderma following photosensitivity caused by hydrochlorothiazide.	**Photodermatol Photoimmunol Photomed**	27	328-330	2011
Kunisada M, Yogianti F, Sakumi K, Ono R, Nakabeppu Y, **Nishigori C**.	Increased Expression of Versican in the Inflammatory Response to UVB- and Reactive Oxygen Species-Induced Skin Tumorigenesis.	**Am J Pathol**	179	3056-3065	2011
Nagai H, Sakaguchi M, Ikeda T, **Nishigori C**.	Grayish brown macules on the axillae, lower abdomen, and groin.	**Arch Dermatol**	147	1097-1102	2011
Kunisada M, Nagai H, Shimizu H, Yogianti F, Nishiyama S, **Nishigori C**.	Double-headed nodules on the abdomen. Myxoid dermatofibrosarcoma protuberans (DFSP).	**Arch Dermatol**	147	857-862	2011

V. 研究成果の刊行物・別刷

IJD | 5372 | B
Journal Name | Manuscript No.
Dispatch: 19.10.11 | Journal: IJD | CE: GeethaPriya P.
Author Received: | No. of pages: 3 | PE: Prasanna
International Journal of Dermatology

Correspondence

Dyschromatosis symmetrica hereditaria by adar1 mutations and viral encephalitis: a hidden link?

Dyschromatosis symmetrica hereditaria (DSH: MIM no. 127400) is an autosomal dominant skin disease caused by a heterozygous mutation of adenosine deaminase acting on RNA 1 gene (*ADAR1*, NM_001111.3), previously termed double-stranded RNA-specific adenosine deaminase gene (*DSRAD*).[1] DSH is characterized by a mixture of hyper- and hypopigmented macules on the dorsal aspects of the extremities. The skin manifestation usually develops during infancy without any known trigger.

Here we report a 4-year-old Hispanic girl who presented with classic DSH following an episode of viral encephalitis. She was healthy and had no skin manifestation until 11 months of age, when she had seizures and developmental delay following symptoms of viral infection, including exanthema over the whole body for several days. She was subsequently diagnosed as having postviral encephalitis. One month after the encephalitis episode, she started to develop the skin manifestations of DSH. At the age of 4, she developed hyper- and hypopigmented macules on the extremities and freckle-like macules on the face (Fig. 1). Mutation search using gDNA eluted from peripheral blood leukocytes revealed that she was heterozygous for the novel mutation c.3102G>A (p.Met1034Ile) in *ADAR1* (Fig. 2).[2] This missense mutation considered to be pathogenic, because the altered amino-acid residue Met1034 is within the deaminase domain of ADAR1 and conserved among diverse species, including zebrafish, frog, chicken, rat, mouse, cow, wolf, and chimpanzee. In addition, the mutation was not detected in the control genomic DNA samples from 102 unrelated, healthy Japanese volunteers.

Two isoforms of human ADAR1 enzymes are known to exist. One is an interferon (IFN)-inducible full-length 150 kDa protein (the p150 isoform), and the other is a constitutively expressed, 110 kDa short isoform (the p110 isoform). Two mutations p.Gln102fs and p.His216fs, which we previously reported in patients with DSH,[3,4] are located at the 5′-side upstream of codon 296 in exon 2, which is the translation initiation codon for ADAR1-p110. Therefore, normal ADAR1-p110 isoform can be produced from the mutant alleles. Thus, DSH is thought to be caused by defects in the p150 isoform.[2] High IFN condition normally induces upregulated expression of ADAR1-p150 isoform in individuals without ADAR1 mutations. This IFN-inducible

COLOR

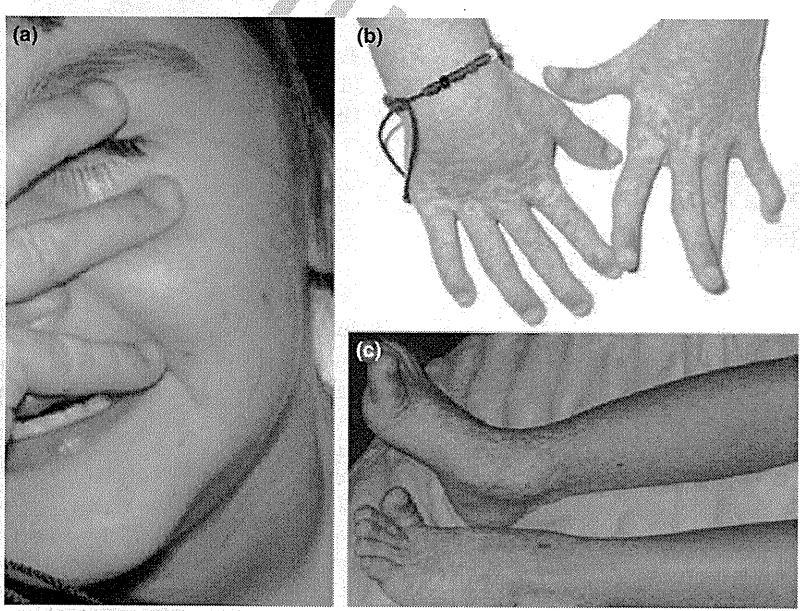

Figure 1 Clinical phenotype of the patient. Freckle-like macules on the face (a), and a mixture of hyper- and hypopigmented macules on the back of the hands (b) and legs (c) were observed

LOW RESOLUTION COLOR FIG

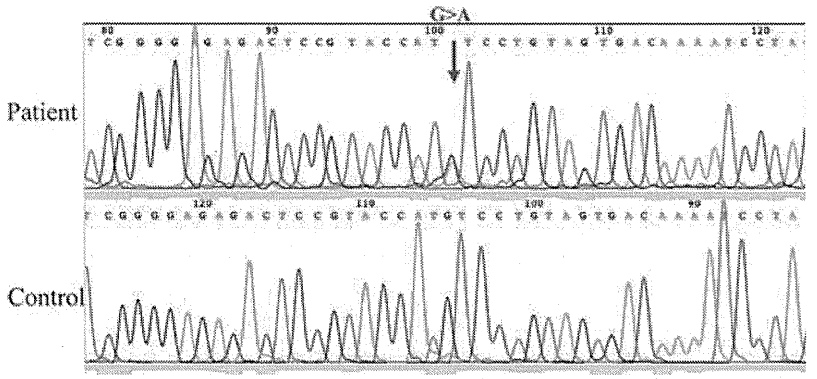

Figure 2 Sequence analysis of *ADAR1* genomic DNA from the patient. The chromatograph reveals c.3102G>A (p.Met1034Ile) in *ADAR1*

isoform is involved in anti-apoptotic pathways[5] and appears to regulate cellular RNA interference (RNAi) efficacy.[6] Thus, under high IFN condition, reduced or absent ADAR1-p150 isoform function might fail to regulate those pathways and be involved in the development of DSH. Further accumulation of data on correlation between DSH and viral infection/high IFN condition will be needed to confirm this hypothesis.

There seems to be no apparent genotype/phenotype correlation in patients with DSH. Carriers with identical mutations can show different clinical features and severity, indicating the contribution of environmental or extrinsic factors on disease development.[2] Viral infection frequently induces increased levels of IFN in the peripheral blood and other tissues. In the present case, it is possible that the high IFN condition induced by systemic viral infection and encephalitis caused the patient's latent shortage of ADAR1 enzymatic ability to become apparent, triggering the development of the DSH phenotype.

Notably, the IFN-inducible p150 isoform of ADAR1 was recently revealed to suppress the replication of paramyxoviruses and orthomyxoviruses.[7] Thus, a loss-of-function mutation of the ADAR1 p150 isoform might be a risk factor for severe infection by measles and other viruses. Further accumulation of cases and experimental data will be needed to elucidate the exact link between DSH with ADAR1 mutations and viral infection.

Acknowledgments

This study was supported in part by grants 21591460 (to M.K.) and 21591459 (to Y.T.) from the Ministry of Education, Science and Culture of Japan.

Michihiro Kono[1], MD, PhD
Masashi Akiyama[1], MD, PhD
Mutsumi Suganuma[1], MD
Yasushi Tomita[1], MD, PhD
[1]Department of Dermatology
Nagoya University Graduate School of Medicine
Nagoya
Japan
E-mail: miro@med.nagoya-u.ac.jp

Amarilis Sanchez-Valle[2], ????
[2]Department of Pediatrics
Division of Genetics
University of South Florida
Tampa
FL
USA

Conflict of interest: None.

doi: 10.1111/j.1365-4632.2011.05372.x

References

1 Miyamura Y, Suzuki T, Kono M, *et al.* Mutations of the RNA-specific adenosine deaminase gene (DSRAD) are involved in dyschromatosis symmetrica hereditaria. *Am J Hum Genet* 2003; 73: 693–699.
2 Kondo T, Suzuki T, Mitsuhashi Y, *et al.* Six novel mutations of the ADAR1 gene in patients with dyschromatosis symmetrica hereditaria: histological observation and comparison of genotypes and clinical phenotypes. *J Dermatol* 2008; 35: 395–406.
3 Suzuki N, Suzuki T, Inagaki K, *et al.* Mutation analysis of the ADAR1 gene in dyschromatosis symmetrica hereditaria

and genetic differentiation from both dyschromatosis universalis hereditaria and acropigmentatio reticularis. *J Invest Dermatol* 2005; **124**: 1186–1192.
4 Suzuki N, Suzuki T, Inagaki K, *et al.* Ten novel mutations of the ADAR1 gene in Japanese patients with dyschromatosis symmetrica hereditaria. *J Invest Dermatol* 2007; **127**: 309–311.
5 Wang Q, Miyakoda M, Yang W, *et al.* Stress-induced apoptosis associated with null mutation of ADAR1 RNA editing deaminase gene. *J Biol Chem* 2004; **279**: 4952–4961.
6 Yang W, Wang Q, Howell KL, *et al.* ADAR1 RNA deaminase limits short interfering RNA efficacy in mammalian cells. *J Biol Chem* 2005; **280**: 3946–3953.
7 Ward SV, George CX, Welch MJ, *et al.* RNA editing enzyme adenosine deaminase is a restriction factor for controlling measles virus replication that also is required for embryogenesis. *Proc Natl Acad Sci USA* 2011; **108**: 331–336.

Author Query Form

Journal: IJD

Article: 5372

Dear Author,
During the copy-editing of your paper, the following queries arose. Please respond to these by marking up your proofs with the necessary changes/additions. Please write your answers on the query sheet if there is insufficient space on the page proofs. Please write clearly and follow the conventions shown on the attached corrections sheet. If returning the proof by fax do not write too close to the paper's edge. Please remember that illegible mark-ups may delay publication.

Many thanks for your assistance.

Query reference	Query	Remarks
1	AUTHOR: passways—do you mean 'pathways' here?	
2	AUTHOR: Please provide the qualification for author Amarilis Sanchez-Valle.	
3	AUTHOR: Sequence analysis of ADAR1 genomic DNA from the patient.—should this also read 'and control' at the end of this sentence?	
4	AUTHOR: Figure 2 has been saved at a low resolution of 273 dpi. Please resupply at 600 dpi. Check required artwork specifications at http://authorservices.wiley.com/submit_illust.asp?site=1	

JAPANESE DERMATOLOGICAL ASSOCIATION
THE JOURNAL OF DERMATOLOGY
doi: 10.1111/j.1346-8138.2011.01385.x
Journal of Dermatology 2011; **38**: 1–3

LETTER TO THE EDITOR

Four novel *ADAR1* gene mutations in patients with dyschromatosis symmetrica hereditaria

Dear Editor,

Dyschromatosis symmetrica hereditaria (DSH; Mendelian Inheritance in Man no. 127400) is a highly penetrant autosomal dominant skin disease and is characterized by a mixture of hyper- and hypopigmented macules on the dorsal aspects of the hand and foot. This disorder commonly develops during infancy or early childhood.[1]

Previous work has shown that a heterozygous mutation of the adenosine deaminase acting on RNA1 gene (*ADAR1* or *DSRAD*) caused DSH in four Japanese DSH families.[2] Subsequently, 115 mutations in the *ADAR1* gene (GenBank accession no. NM_001111.3, http://www.ncbi.nlm.nih.gov/genbank/) have been reported in Japanese, Taiwanese and Chinese patients with DSH. The ADAR1 protein catalyzes the deamination of adenosine to inosine in double-stranded RNA.[3] This creates alternative splice sites or alterations of the codon that lead to functional changes in the target substrate(s). However, the target gene(s) for ADAR1 in the skin as well as the mechanisms by which mutations in *ADAR1* cause DSH remain unknown. In this study, we performed mutational analysis of the *ADAR1* gene in three Japanese and a Chinese Canadian with DSH.

The analysis was performed as follows. Genomic DNA was extracted from peripheral blood with a QIAamp DNA blood maxi kit (QIAGEN, Valencia, CA, USA) and was used as a template for mutational screening with a polymerase chain reaction (PCR)-based single-strand conformation polymorphism (SSCP)/heteroduplex (HD) analysis.[4] Standard PCR amplification procedures were employed with high fidelity polymerase, Platinum Taq DNA Polymerase (Invitrogen, Carlsbad, CA, USA) and an annealing temperature of 62°C for all primers.[5] SSCP gel with glycerol concentrations of 7.5% was used. PCR products showing aberrant patterns on SSCP were re-amplified and sequenced directly to identify a mutation. In patients without any mutation detected by the SSCP/HD method, all of their PCR products were directly sequenced to identify mutations. Informed consent and blood samples of patients were obtained under protocols approved by the Ethics Committee of Nagoya University School of Medicine. Three Japanese patients in this study were not related to each other.

We identified four novel heterozygous *ADAR1* mutations including one splice mutation (IVS9-1G>T) and three frame-shift mutations (p.Lys1167fsX1178, p.Asn398fsX401 and p.Asp1147fsX1152) (Table 1).

The three frame-shift mutations code a different amino acid sequence and eventually make a new stop codon. In a previous report,[6] the ADAR1 mutations p.Gln513X and p.Cys519fs, which lead to premature termination codon (PTC) and to nonsense-mediated mRNA decay (NMD), were reported to result in ADAR1 haploinsufficiency. Generally, PTC could initiate NMD as long as the stop codons are located more than 50–55 nucleotides upstream of the 3′-most splice site.[7] We speculate that p.Asn398fsX401 on exon 2 of 15 exons in the present patient would also lead to haploinsufficiency of ADAR1 enzyme activity. The other two frame-shift mutations made PTC in exon 15. Thus, these mutations would not result in NMD, but made aberrant proteins.

On the other hand, the site of the novel splice mutation (IVS9-1G>T) was the conserved sequence on splice accepter site on intron 9 (Fig. 1a). On the *ADAR1* gene of a DSH patient some splice mutations have been reported.[8] So far, only one investigation on aberrant splicing of the *ADAR1* gene by the splice mutation has been reported.[5] We investigated aberrant splicing of the mutation IVS9-1G>T. Total RNA was extracted from whole blood of patient 4 and a healthy control.

Reverse transcription PCR was done for amplification of the 172-bp fragment at the boundary between exon 9 and 10 on mRNA from whole blood of the patient. Extra and normal bands, 172 bp (normal product) and 160 bp (aberrantly splicing product), were confirmed in mRNA from the patient (Fig. 1b). These were separated by agarose gel extraction and subcloned each with a TOPO TA Cloning kit for Sequencing (Invitrogen). DNA sequencing of subcloned PCR products showed the truncation of the 12-bp nucleotides

Table 1. Four mutations of the *ADAR1* gene in the patient with dyschromatosis symmetrica hereditaria

Patient no.	Patient			Mutation		
	Race	Onset	Complication	Nucleotide change†	Amino acid change	Position
1	Japanese	3 years old	Healthy	c.3499del A	p.Lys1167fsX1178	Exon 15
2	Japanese	2 years old	Healthy	c.1192-3 del AA	p.Asn398fsX401	Exon 2
3	Chinese Canadian	At birth	Healthy	c.3440insG	p.Asp1147fsX1152	Exon 14
4	Japanese	6 years old	Febrile seizure at 2 year-old	c.IVS9-1 G>T	p.Arg921_Tyr924del	IVS9

†GenBank accession no. NM_001111.3. Position 1 is A of the translation initiation codon.

Correspondence: Michihiro Kono, M.D., Ph.D., Department of Dermatology, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan. Email: miro@med.nagoya-u.ac.jp

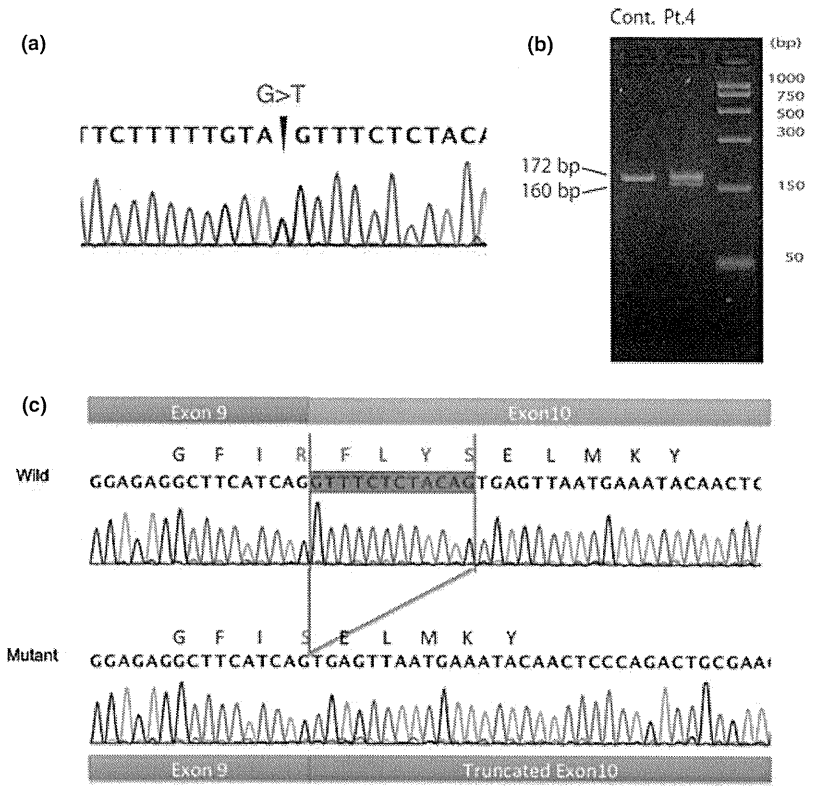

Figure 1. Molecular analysis of splicing mutation on *ADAR1* of patient 4. (a) Sequence analysis of boundary between exons 9 and 10 on *ADAR1* genomic DNA from patient 4 reveals G to T transversion, IVS9-1G>T. (b) Agarose gel electrophoresis of reverse transcription polymerase chain reaction (RT–PCR) products of boundary between exons 9 and 10 on *ADAR1* gene. The RT–PCR product from the patient 4 shows 172-bp (normally spliced fragment) and 160-bp (aberrantly spliced fragment) bands, although a control specimen exhibits only a 172-bp band. (c) Sequence analysis of boundary between exons 9 and 10 on *ADAR1* cDNA. In the mutant cDNA, the aberrant splicing results in 12-bp deletion.

on the 5′-side of exon 10 (Fig. 1c). Subsequently, the deletion of four amino acids (p.Arg921-Tyr924del) was in the deaminase domain and should produce the aberrant protein of ADAR1.

All patients phenotypically presented typical macules on the dorsal aspects of the hands and feet, lower arms and lower legs. No patients had complications but patient 4 had a febrile seizure at 2 years old. The mutations reported in the published work including the present report are scattered through the entire gene and there is no hotspot for the *ADAR1* gene mutations. However, in more than 100 mutations on *ADAR1*, all missense mutations except for p.Arg26Lys were in the adenosine–deaminase domain. Thus, this domain is thought to be essential for the biochemical function of ADAR1. There is no apparent genotype/phenotype correlation in *ADAR1* mutations. Even in one family, the identical mutation was reported to lead to different phenotypes.[5] The reason why a low activity of ADAR1 induces the peculiar skin lesions localized on the extremities is still unknown. We speculate that, when melanoblasts migrate from the neural crest to the skin during development, insufficient ADAR1 enzyme activity might affect differentiation of melanoblasts to melanocytes and the melanoblasts located distant from the neural crest might be most seriously affected during migration.[9]

In conclusion, we reported four novel mutations in the *ADAR1* genes of DSH patients. The accumulation of the patients with genetic information may provide new insight into the pathogenesis of DSH.

ACKNOWLEDGMENTS

We are grateful to the patients, their families and volunteers for providing blood samples. This work was supported by grants 21591460 (M. K.) and 21591459 (Y. T.) from the Ministry of Education, Science and Culture of Japan.

Michihiro KONO,[1] Masashi AKIYAMA,[1] Taisuke KONDO,[1] Tamio SUZUKI,[2] Mutsumi SUGANUMA,[1] Mari WATAYA-KANEDA,[3] Joseph LAM,[4] Akihiko SHIBAKI,[5] Yasushi TOMITA[1]

[1]*Department of Dermatology, Nagoya University Graduate School of Medicine, Nagoya,* [2]*Department of Dermatology, Yamagata University School of Medicine, Yamagata,* [3]*Department of Dermatology, Osaka University Graduate School of Medicine, Suita,* [5]*Department of Dermatology, Hokkaido University Graduate School of Medicine, Sapporo, Japan; and* [4]*Department of Pediatrics and Department of Dermatology, University of British Columbia, Vancouver, British Columbia, Canada*

REFERENCES

1 Kono M, Miyamura Y, Matsunaga J, Tomita Y. Exclusion of linkage between dyschromatosis symmetrica hereditaria and chromosome 9. *J Dermatol Sci* 2000; **22**: 88–95.

2 Miyamura Y, Suzuki T, Kono M *et al.* Mutations of the RNA-specific adenosine deaminase gene (DSRAD) are involved in dyschromatosis symmetrica hereditaria. *Am J Hum Genet* 2003; **73**: 693–699.

3 Bass BL, Weintraub H. An unwinding activity that covalently modifies its double-stranded RNA substrate. *Cell* 1988; **55**: 1089–1098.

4 Spritz RA, Holmes SA, Ramesar R, Greenberg J, Curtis D, Beighton P. Mutations of the KIT (mast/stem cell growth factor receptor) proto-oncogene account for a continuous range of phenotypes in human piebaldism. *Am J Hum Genet* 1992; **51**: 1058–1065.

5 Suzuki N, Suzuki T, Inagaki K *et al.* Mutation analysis of the ADAR1 gene in dyschromatosis symmetrica hereditaria and genetic differentiation from both dyschromatosis universalis hereditaria and acropigmentatio reticularis. *J Invest Dermatol* 2005; **124**: 1186–1192.

6 Liu Q, Jiang L, Liu WL *et al.* Two novel mutations and evidence for haploinsufficiency of the ADAR gene in dyschromatosis symmetrica hereditaria. *Br J Dermatol* 2006; **154**: 636–642.

7 Nagy E, Maquat LE. A rule for termination-codon position within intron-containing genes: when nonsense affects RNA abundance. *Trends Biochem Sci* 1998; **23**: 198–199.

8 Wang XP, Liu Y, Wang JM, Xiao SX. Two novel splice site mutations of the ADAR1 gene in Chinese families with dyschromatosis symmetrica hereditaria. *J Dermatol* 2010; **37**: 1051–1052.

9 Tomita Y, Suzuki T. Genetics of pigmentary disorders. *Am J Med Genet C* 2004; **131C**: 75–81.