

Table XIV. Examples of foundations, patient organizations, and useful Internet links

Foundations and registries	
United States: Foundation for Ichthyosis and Related Skin Types (www.scalyskin.org), Registry for Ichthyosis and Related Disorders (www.skinregistry.org)	
Germany (Europe): Network for Ichthyoses and Related Keratinization Disorders (www.netzwerk-ichthyose.de/)	
Japan: Registry for Autosomal Recessive Congenital Ichthyosis and Keratinopathic Ichthyosis supported by Health and Labor Science Research Grants, Research on Intractable Diseases, Ministry of Health, Labor, and Welfare	
Austria: National Registry for Genodermatoses Including Ichthyoses	
Patient organizations for ichthyosis	
Austria	www.selbsthilfe-tirol.at/Selbsthilfegruppen/Gruppen/Ichthyose.htm
Belgium	www.devidts.com/ichthyosis
Denmark	www.iktyosis.dk
Finland	www.iholiitto.fi/
France	www.anips.net/
Germany	www.ichthyose.de
Italy	www.ittiosi.it/
Japan	www.gyorinsen.com
Monaco	www.aaimonaco.org
Spain	www.ictiosis.org
Sweden	www.iktyos.nu//
Switzerland	www.ichthyose.ch
United Kingdom	www.ichthyosis.org.uk/
United States	www.scalyskin.org
Other databases and Internet links	
World Wide Web site hosted at National Center for Biotechnology Information (NCBI):	www.genetests.org
Portal for rare diseases and orphan drugs:	www.orpha.net
Human intermediated filament database:	www.interfil.org
German guidelines for diagnosis and treatment of ichthyoses:	www.uni-duesseldorf.de/AWMF/II/013-043.htm

corresponds to the diagnostic low-sulfur protein content of the hair.^{219,220} Special immunohistochemical procedures can be combined, eg, to confirm filaggrin deficiency in IV,^{202,221} or demonstrate absent or reduced expression of LEKTI that supports the diagnosis of NS.²²²⁻²²⁴ To screen for TGase-1 deficiency in ARCI unfixed cryostat sections are used for the enzyme activity assay.^{225,226} Alternatively, superficial SC material can be subjected to a SDS heating test that visualizes absent cross-linked envelopes in TGase-1 deficiency.²²⁷

There are special useful analyses given in Tables IV to XII. For instance, steroid sulfatase deficiency underlying RXLI can be demonstrated by reduced arylsulfatase-C activity of leukocytes, or can readily be diagnosed by the widely available fluorescent in situ hybridization test for the STS gene region, because more than 90% of the cases are caused by a gene deletion. Gas chromatography-mass spectrometry reveals elevated serum levels of 8-dehydrocholesterol and cholesterol in Conradi-Hünemann-Happle syndrome and can identify a somatic *EBP* gene mosaicism in unaffected individuals.²²⁸

RESOURCES FOR CLINICIANS AND PATIENTS

Currently, therapy of most ichthyoses is neither type-specific nor corrective, but rather its goal is to relieve symptoms.^{6,35,46,229-232} Importantly, clinicians have to consider the functional consequences of the epidermal barrier defect, such as increased risk of systemic absorption and toxicity, especially in infants.²³¹⁻²³³ Neonates with severe congenital phenotypes may require intensive care using humidified isolettes (incubators) to avoid temperature instability and hypernatremic dehydration, and observation for signs of cutaneous infection and septicemia. Caloric insufficiency as a result of evaporative energy losses places infants with severe phenotypes at risk for growth failure and requires early intervention.^{234,235}

Affected individuals and/or their families should be offered genetic counseling to explain the nature of the disorder, its mode of inheritance, and the probability of future disease manifestations in the family.^{1,3} They should be offered psychologic support and be informed of patient organizations or foundations (Table XIV).

We would like to dedicate this classification to all our patients and their families, and thank all colleagues and friends, who are helping to achieve optimal clinical care for affected individuals and/or promote through their research our knowledge about the disorders of cornification. We are deeply grateful for the generous financial support of the Laboratories Pierre Fabre, and would like to say "grand merci" to Anita Couteau, Didier Coustou, and Pascal Lefrancois—and to Brigitte Willis from the Network for Ichthyoses and Related Keratinization Disorders Center in Münster, who together perfectly organized the wonderful, unforgettable conference in Sorèze. Moreover, we would like to acknowledge the help of Dr Dan Ben Amitai and Dr Hagen Ott for providing photographs, and Jutta Bückmann for the help with the slides from the Department of Dermatology, Münster (head Thomas A. Luger). We also express gratitude to Meral Arin, Steffen Emmert, Rudolf Happle, Peter Höger, and Dieter Metzke for their support and helpful comments. The first author wants to thank his wonderful family, namely Melody, Alanna, and Amechi.

REFERENCES

- Williams ML, Elias PM. Ichthyosis: genetic heterogeneity, genodermatoses, and genetic counseling. *Arch Dermatol* 1986;122:529-31.
- Willan R. *On cutaneous diseases*. London: Barnard; 1808.
- Traupe H. *The ichthyoses: a guide to clinical diagnosis, genetic counseling, and therapy*. Berlin: Springer Verlag; 1989.
- Akiyama M, Shimizu H. An update on molecular aspects of the non-syndromic ichthyoses. *Exp Dermatol* 2008;17:373-82.
- DiGiovanna JJ, Robinson-Bostom L. Ichthyosis: etiology, diagnosis, and management. *Am J Clin Dermatol* 2003;4:81-95.
- DiGiovanna JJ. Ichthyosiform dermatoses: so many discoveries, so little progress. *J Am Acad Dermatol* 2004;51(Suppl):S31-4.
- Elias PM, Williams ML, Holleran WM, Jiang YJ, Schmuth M. Thematic review series: skin lipids. Pathogenesis of permeability barrier abnormalities in the ichthyoses: inherited disorders of lipid metabolism. *J Lipid Res* 2008;49:697-714.
- Elias PM, Williams ML, Crumrine D, Schmuth M. *Ichthyoses - Clinical, Biochemical, Pathogenic and Diagnostic Assessment*. Karger. In press.
- Oji V, Traupe H. Ichthyoses: differential diagnosis and molecular genetics. *Eur J Dermatol* 2006;16:349-59.
- Richard G. Molecular genetics of the ichthyoses. *Semin Med Genet* 2004;131C:32-44.
- Schmuth M, Gruber R, Elias PM, Williams ML. Ichthyosis update: towards a function-driven model of pathogenesis of the disorders of cornification and the role of corneocyte proteins in these disorders. *Adv Dermatol* 2007;23:231-56.
- Siemens HW. Die Vererbung in der Ätiologie der Hautkrankheiten. In: Jadassohn J, editor. *Handbuch der Haut- und Geschlechtskrankheiten* 3. Berlin: Springer Verlag; 1929. pp. 1-165.
- Schnyder UW. Inherited ichthyoses. *Arch Dermatol* 1970;102:240-52.
- Riecke E. Über ichthyosis congenita. *Arch Dermatol Syph* 1900;54:289-340.
- Kelsell DP, Norgett EE, Unsworth H, Teh MT, Cullup T, Mein CA, et al. Mutations in ABCA12 underlie the severe congenital skin disease harlequin ichthyosis. *Am J Hum Genet* 2005;76:794-803.
- Akiyama M, Sugiyama-Nakagiri Y, Sakai K, McMillan JR, Goto M, Arita K, et al. Mutations in lipid transporter ABCA12 in harlequin ichthyosis and functional recovery by corrective gene transfer. *J Clin Invest* 2005;115:1777-84.
- Lefevre C, Audebert S, Jobard F, Bouadjar B, Lakhdar H, Boughdene-Stambouli O, et al. Mutations in the transporter ABCA12 are associated with lamellar ichthyosis type 2. *Hum Mol Genet* 2003;12:2369-78.
- Parmentier L, Lakhdar H, Blanchet-Bardon C, Marchand S, Dubertret L, Weissenbach J. Mapping of a second locus for lamellar ichthyosis to chromosome 2q33-35. *Hum Mol Genet* 1996;5:555-9.
- Natsuga K, Akiyama M, Kato N, Sakai K, Sugiyama-Nakagiri Y, Nishimura M, et al. Novel ABCA12 mutations identified in two cases of non-bullous congenital ichthyosiform erythroderma associated with multiple skin malignant neoplasia. *J Invest Dermatol* 2007;127:2669-73.
- Sakai K, Akiyama M, Yanagi T, McMillan JR, Suzuki T, Tsukamoto K, et al. ABCA12 is a major causative gene for non-bullous congenital ichthyosiform erythroderma. *J Invest Dermatol* 2009;129:2306-9.
- Lawlor F. Progress of a harlequin fetus to nonbullous ichthyosiform erythroderma. *Pediatrics* 1988;82:870-3.
- Huber M, Rettler I, Bernasconi K, Frenk E, Lavrijsen SP, Ponc M, et al. Mutations of keratinocyte transglutaminase in lamellar ichthyosis. *Science* 1995;267:525-8.
- Russell LJ, DiGiovanna JJ, Rogers GR, Steinert PM, Hashem N, Compton JG, et al. Mutations in the gene for transglutaminase 1 in autosomal recessive lamellar ichthyosis. *Nat Genet* 1995;9:279-83.
- Lefevre C, Bouadjar B, Karaduman A, Jobard F, Saker S, Ozguc M, et al. Mutations in ichthyin a new gene on chromosome 5q33 in a new form of autosomal recessive congenital ichthyosis. *Hum Mol Genet* 2004;13:2473-82.
- Lefevre C, Bouadjar B, Ferrand V, Tadini G, Megarbane A, Lathrop M, et al. Mutations in a new cytochrome P450 gene in lamellar ichthyosis type 3. *Hum Mol Genet* 2006;15:767-76.
- Jobard F, Lefevre C, Karaduman A, Blanchet-Bardon C, Emre S, Weissenbach J, et al. Lipoyxygenase-3 (ALOXE3) and 12(R)-lipoyxygenase (ALOX12B) are mutated in non-bullous congenital ichthyosiform erythroderma (NCIE) linked to chromosome 17p13.1. *Hum Mol Genet* 2002;11:107-13.
- Fischer J. Autosomal recessive congenital ichthyosis. *J Invest Dermatol* 2009;129:1319-21.
- Eckl KM, de Juanes S, Kurtenbach J, Natebus M, Lugassy J, Oji V, et al. Molecular analysis of 250 patients with autosomal recessive congenital ichthyosis: evidence for mutation hotspots in ALOXE3 and allelic heterogeneity in ALOX12B. *J Invest Dermatol* 2009;129:1421-8.
- Hatsell SJ, Stevens H, Jackson AP, Kelsell DP, Zvulunov A. An autosomal recessive exfoliative ichthyosis with linkage to chromosome 12q13. *Br J Dermatol* 2003;149:174-80.
- Mizrachi-Koren M, Geiger D, Indelman M, Bitterman-Deutsch O, Bergman R, Sprecher E. Identification of a novel locus associated with congenital recessive ichthyosis on 12p11.2-q13. *J Invest Dermatol* 2005;125:456-62.
- Akiyama M, Sawamura D, Shimizu H. The clinical spectrum of nonbullous congenital ichthyosiform erythroderma and lamellar ichthyosis. *Clin Exp Dermatol* 2003;28:235-40.
- Arita K, Jacyk WK, Wessagowit V, van Rensburg EJ, Chaplin T, Mein CA, et al. The South African "bathing suit ichthyosis" is a form of lamellar ichthyosis caused by a homozygous missense mutation, p.R315L, in transglutaminase 1. *J Invest Dermatol* 2007;127:490-3.

33. Dahlqvist J, Klar J, Hausser I, Anton-Lamprecht I, Pigg MH, Gedde-Dahl T Jr, et al. Congenital ichthyosis: mutations in ichthyin are associated with specific structural abnormalities in the granular layer of epidermis. *J Med Genet* 2007;44: 615-20.
34. Eckl KM, Krieg P, Kuster W, Traupe H, Andre F, Wittstruck N, et al. Mutation spectrum and functional analysis of epidermis-type lipoygenases in patients with autosomal recessive congenital ichthyosis. *Hum Mutat* 2005;26:351-61.
35. Farasat S, Wei MH, Herman M, Liewehr DJ, Steinberg SM, Bale SJ, et al. Novel transglutaminase-1 mutations and genotype-phenotype investigations of 104 patients with autosomal recessive congenital ichthyosis in the USA. *J Med Genet* 2009; 46:103-11.
36. Frenk E. A spontaneously healing collodion baby: a light and electron microscopical study. *Acta Derm Venereol* 1981;61: 168-71.
37. Harting M, Brunetti-Pierri N, Chan CS, Kirby J, Dishop MK, Richard G, et al. Self-healing collodion membrane and mild nonbullous congenital ichthyosiform erythroderma due to 2 novel mutations in the ALOX12B gene. *Arch Dermatol* 2008; 144:351-6.
38. Hennies HC, Kuster W, Wiebe V, Krebsova A, Reis A. Genotype/phenotype correlation in autosomal recessive lamellar ichthyosis. *Am J Hum Genet* 1998;62:1052-61.
39. Herman ML, Farasat S, Steinbach PJ, Wei MH, Toure O, Fleckman P, et al. Transglutaminase-1 gene mutations in autosomal recessive congenital ichthyosis: summary of mutations (including 23 novel) and modeling of TGase-1. *Hum Mutat* 2009;30:537-47.
40. Lefevre C, Jobard F, Caux F, Bouadjar B, Karaduman A, Heilig R, et al. Mutations in CGI-58, the gene encoding a new protein of the esterase/lipase/thioesterase subfamily, in Chanarin-Dorfman syndrome. *Am J Hum Genet* 2001;69:1002-12.
41. Mazereeuw-Hautier J, Aufenvenne K, Deraison C, Ahvazi B, Oji V, Traupe H, et al. Acral self-healing collodion baby: report of a new clinical phenotype caused by a novel TGM1 mutation. *Br J Dermatol* 2009;161:456-63.
42. Oji V, Hautier JM, Ahvazi B, Hausser I, Aufenvenne K, Walker T, et al. Bathing suit ichthyosis is caused by transglutaminase-1 deficiency: evidence for a temperature-sensitive phenotype. *Hum Mol Genet* 2006;15:3083-97.
43. Petit E, Huber M, Rochat A, Bodemer C, Teillac-Hamel D, Muh JP, et al. Three novel point mutations in the keratinocyte transglutaminase (TGK) gene in lamellar ichthyosis: significance for mutant transcript level, TGK immunodetection and activity. *Eur J Hum Genet* 1997;5:218-28.
44. Raghunath M, Hennies HC, Ahvazi B, Vogel M, Reis A, Steinert PM, et al. Self-healing collodion baby: a dynamic phenotype explained by a particular transglutaminase-1 mutation. *J Invest Dermatol* 2003;120:224-8.
45. Vahlquist A, Ganemo A, Pigg M, Virtanen M, Westermarck P. The clinical spectrum of congenital ichthyosis in Sweden: a review of 127 cases. *Acta Derm Venereol Suppl (Stockh)* 2003;83(3):34-47.
46. Vahlquist A, Ganemo A, Virtanen M. Congenital ichthyosis: an overview of current and emerging therapies. *Acta Derm Venereol* 2008;88:4-14.
47. Jacyk WK. Bathing-suit ichthyosis: a peculiar phenotype of lamellar ichthyosis in South African blacks. *Eur J Dermatol* 2005;15:433-6.
48. Aufenvenne K, Oji V, Walker T, Becker-Pauly C, Hennies HC, Stocker W, et al. Transglutaminase-1 and bathing suit ichthyosis: molecular analysis of gene/environment interactions. *J Invest Dermatol* 2009;129:2068-71.
49. Reed WB, Herwick RP, Harville D, Porter PS, Conant M. Lamellar ichthyosis of the newborn: a distinct clinical entity; its comparison to the other ichthyosiform erythrodermas. *Arch Dermatol* 1972;105:394-9.
50. Anton-Lamprecht I. Prenatal diagnosis of genetic disorders of the skin by means of electron microscopy. *Hum Genet* 1981; 59:392-405.
51. Anton-Lamprecht I. Genetically induced abnormalities of epidermal differentiation and ultrastructure in ichthyoses and epidermolyses: pathogenesis, heterogeneity, fetal manifestation, and prenatal diagnosis. *J Invest Dermatol* 1983;81:149-56.
52. Frost P, Weinstein GD, Van Scott EJ. The ichthyosiform dermatoses, II: autoradiographic studies of epidermal proliferation. *J Invest Dermatol* 1966;47:561-7.
53. Ishida-Yamamoto A, McGrath JA, Judge MR, Leigh IM, Lane EB, Eady RA. Selective involvement of keratins K1 and K10 in the cytoskeletal abnormality of epidermolytic hyperkeratosis (bullous congenital ichthyosiform erythroderma). *J Invest Dermatol* 1992;99:19-26.
54. Ishida-Yamamoto A, Takahashi H, Iizuka H. Immunoelectron microscopy links molecules and morphology in the studies of keratinization. *Eur J Dermatol* 2000;10:429-35.
55. Lapière S. Epidermolyse ichthyosiforme congénitale (erythrodermie ichthyosiforme congénitale forme bulleuse de Brocq). *Ann Dermatol Syph* 1932;3:401-15.
56. Grimberg G, Hausser I, Muller FB, Wodecki K, Schaffrath C, Krieg T, et al. Novel and recurrent mutations in the 1B domain of keratin 1 in palmoplantar keratoderma with tonotubules. *Br J Dermatol* 2009;160:446-9.
57. Ishida-Yamamoto A, Richard G, Takahashi H, Iizuka H. In vivo studies of mutant keratin 1 in ichthyosis hystrix Curth-Macklin. *J Invest Dermatol* 2003;120:498-500.
58. Ollendorff-Curth H, Allen FH Jr, Schnyder UW, Anton-Lamprecht I. Follow-up of a family group suffering from ichthyosis hystrix type Curth-Macklin. *Humangenetik* 1972;17:37-48.
59. Sprecher E, Ishida-Yamamoto A, Becker OM, Marekov L, Miller CJ, Steinert PM, et al. Evidence for novel functions of the keratin tail emerging from a mutation causing ichthyosis hystrix. *J Invest Dermatol* 2001;116:511-9.
60. Schmuth M, Yosipovitch G, Williams ML, Weber F, Hintner H, Ortiz-Urda S, et al. Pathogenesis of the permeability barrier abnormality in epidermolytic hyperkeratosis. *J Invest Dermatol* 2001;117:837-47.
61. Traupe H, Kolde G, Hamm H, Happle R. Ichthyosis bullosa of Siemens: a unique type of epidermolytic hyperkeratosis. *J Am Acad Dermatol* 1986;14:1000-5.
62. DiGiovanna JJ, Bale SJ. Clinical heterogeneity in epidermolytic hyperkeratosis. *Arch Dermatol* 1994;130:1026-35.
63. Arin MJ. The molecular basis of human keratin disorders. *Hum Genet* 2009;125:355-73.
64. Bale SJ, DiGiovanna JJ. Genetic approaches to understanding the keratinopathies. *Adv Dermatol* 1997;12:99-113.
65. DiGiovanna JJ, Bale SJ. Epidermolytic hyperkeratosis: applied molecular genetics. *J Invest Dermatol* 1994;102:390-4.
66. Morais P, Mota A, Baudrier T, Lopes JM, Cerqueira R, Tavares P, et al. Epidermolytic hyperkeratosis with palmoplantar keratoderma in a patient with KRT10 mutation. *Eur J Dermatol* 2009;19:333-6.
67. Muller FB, Huber M, Kinaciyan T, Hausser I, Schaffrath C, Krieg T, et al. A human keratin 10 knockout causes recessive epidermolytic hyperkeratosis. *Hum Mol Genet* 2006;15:1133-41.
68. Curth H, Macklin MT. The genetic basis of various types of ichthyosis in a family group. *Am J Hum Genet* 1954;6:371-82.
69. Jan AY, Amin S, Ratajczak P, Richard G, Sybert VP. Genetic heterogeneity of KID syndrome: identification of a Cx30 gene

- (GJB6) mutation in a patient with KID syndrome and congenital atrichia. *J Invest Dermatol* 2004;122:1108-13.
70. Joh GY, Traupe H, Metzke D, Nashan D, Huber M, Hohl D, et al. A novel dinucleotide mutation in keratin 10 in the annular epidermolytic ichthyosis variant of bullous congenital ichthyosiform erythroderma. *J Invest Dermatol* 1997;108:357-61.
 71. Sybert VP, Francis JS, Corden LD, Smith LT, Weaver M, Stephens K, et al. Cyclic ichthyosis with epidermolytic hyperkeratosis: a phenotype conferred by mutations in the 2B domain of keratin K1. *Am J Hum Genet* 1999;64:732-8.
 72. Nazzaro V, Ermacora E, Santucci B, Caputo R. Epidermolytic hyperkeratosis: generalized form in children from parents with systematized linear form. *Br J Dermatol* 1990;122:417-22.
 73. Paller AS, Syder AJ, Chan YM, Yu QC, Hutton E, Tadini G, et al. Genetic and clinical mosaicism in a type of epidermal nevus. *N Engl J Med* 1994;331:1408-15.
 74. Tsubota A, Akiyama M, Sakai K, Goto M, Nomura Y, Ando S, et al. Keratin 1 gene mutation detected in epidermal nevus with epidermolytic hyperkeratosis. *J Invest Dermatol* 2007;127:1371-4.
 75. De Laurenzi V, Rogers GR, Hamrock DJ, Marekov LN, Steinert PM, Compton JG, et al. Sjögren-Larsson syndrome is caused by mutations in the fatty aldehyde dehydrogenase gene. *Nat Genet* 1996;12:52-7.
 76. Sjögren T, Larsson T. Oligophrenia in combination with congenital ichthyosis and spastic disorders: a clinical and genetic study. *Acta Psychiatr Neurol Scand* 1957;32:1-112s.
 77. Refsum S, Salomonsen L, Skatvedt M. Heredopathia atactica polyneuritiformis in children. *J Pediatr* 1949;35:335-43.
 78. Reed WB, Stone VM, Boder E, Ziprkowski L. Hereditary syndromes with auditory and dermatological manifestations. *Arch Dermatol* 1967;95:456-61.
 79. Chanarin I, Patel A, Slavin G, Wills EJ, Andrews TM, Stewart G. Neutral-lipid storage disease: a new disorder of lipid metabolism. *Br Med J* 1975;1:553-5.
 80. Dorfman ML, Hershko C, Eisenberg S, Sagher F. Ichthyosiform dermatosis with systemic lipidosis. *Arch Dermatol* 1974;110:261-6.
 81. MacLeod JMH. Three cases of 'ichthyosis follicularis' associated with baldness. *Br J Dermatol* 1909;21:165-89.
 82. Hamm H, Meinecke P, Traupe H. Further delineation of the ichthyosis follicularis, atrichia, and photophobia syndrome. *Eur J Pediatr* 1991;150:627-9.
 83. Happle R. X-linked dominant chondrodysplasia punctata: review of literature and report of a case. *Hum Genet* 1979;53:65-73.
 84. Braverman N, Lin P, Moebius FF, Obie C, Moser A, Glossmann H, et al. Mutations in the gene encoding 3 beta-hydroxysteroid-delta 8, delta 7-isomerase cause X-linked dominant Conradi-Hünermann syndrome. *Nat Genet* 1999;22:291-4.
 85. Castano SE, Segurado RA, Guerra TA, Simon de las HR, Lopez-Rios F, Coll Rosell MJ. Ichthyosis: the skin manifestation of multiple sulfatase deficiency. *Pediatr Dermatol* 1997;14:369-72.
 86. Dierks T, Schmidt B, Borissenko LV, Peng J, Preusser A, Mariappan M, et al. Multiple sulfatase deficiency is caused by mutations in the gene encoding the human C(alpha)-formylglycine generating enzyme. *Cell* 2003;113:435-44.
 87. Happle R, Kuster W. Ichthyosis variegata: a new name for a neglected disease. *J Am Acad Dermatol* 1997;36:500.
 88. Brusasco A, Tadini G, Cambiaghi S, Ermacora E, Grimalt R, Caputo R. A case of congenital reticular ichthyosiform erythroderma—ichthyosis 'en confettis'. *Dermatology* 1994;188:40-5.
 89. Bygum A, Westermark P, Brandrup F. Ichthyosis prematurity syndrome: a well-defined congenital ichthyosis subtype. *J Am Acad Dermatol* 2008;59(Suppl):S71-4.
 90. Klar J, Schweiger M, Zimmerman R, Zechner R, Li H, Torma H, et al. Mutations in the fatty acid transport protein 4 gene cause the ichthyosis prematurity syndrome. *Am J Hum Genet* 2009;85:248-53.
 91. Phadnis SV, Griffin DR, Eady RA, Rodeck CH, Chitty LS. Prenatal diagnosis and management strategies in a family with a rare type of congenital ichthyosis. *Ultrasound Obstet Gynecol* 2007;30:908-10.
 92. Faghri S, Tamura D, Kraemer KH, DiGiovanna JJ. Trichothiodystrophy: a systematic review of 112 published cases characterizes a wide spectrum of clinical manifestations. *J Med Genet* 2008;45:609-21.
 93. Kraemer KH, Patronas NJ, Schiffmann R, Brooks BP, Tamura D, DiGiovanna JJ. Xeroderma pigmentosum, trichothiodystrophy and Cockayne syndrome: a complex genotype-phenotype relationship. *Neuroscience* 2007;145:1388-96.
 94. Morice-Picard F, Cario-André M, Rezvani H, Sarasin A, Lacombe D, Taieb A. New clinico-genetic classification of trichothiodystrophy. *Am J Med Genet* 2009;149A:2020-30.
 95. Camisa C, Hessel A, Rossana C, Parks A. Autosomal dominant keratoderma, ichthyosiform dermatosis and elevated serum beta-glucuronidase. *Dermatologica* 1988;177:341-7.
 96. Korge BP, Ishida-Yamamoto A, Punter C, Dopping-Hepenstal PJ, Iizuka H, Stephenson A, et al. Loricrin mutation in Vohwinkel's keratoderma is unique to the variant with ichthyosis. *J Invest Dermatol* 1997;109:604-10.
 97. Maestrini E, Monaco AP, McGrath JA, Ishida-Yamamoto A, Camisa C, Hovnanian A, et al. A molecular defect in loricrin, the major component of the cornified cell envelope, underlies Vohwinkel's syndrome. *Nat Genet* 1996;13:70-7.
 98. Sprecher E, Ishida-Yamamoto A, Mizrahi-Koren M, Rapaport D, Goldsher D, Indelman M, et al. A mutation in SNAP29, coding for a SNARE protein involved in intracellular trafficking, causes a novel neurocutaneous syndrome characterized by cerebral dysgenesis, neuropathy, ichthyosis, and palmo-plantar keratoderma. *Am J Hum Genet* 2005;77:242-51.
 99. Gissen P, Johnson CA, Morgan NV, Stapelbroek JM, Forshev T, Cooper WN, et al. Mutations in VPS33B, encoding a regulator of SNARE-dependent membrane fusion, cause arthrogyrosis—renal dysfunction—cholestasis (ARC) syndrome. *Nat Genet* 2004;36:400-4.
 100. Lutz-Richner AR, Landolt RF. Familial bile duct malformation with tubular renal insufficiency (Familiäre Gallengansmissbildungen mit tubularer Neiereninsuffizienz). *Helv Paediatr Acta* 1973;28:1-12.
 101. Jang JY, Kim KM, Kim GH, Yu E, Lee JJ, Park YS, et al. Clinical characteristics and VPS33B mutations in patients with ARC syndrome. *J Pediatr Gastroenterol Nutr* 2009;48:348-54.
 102. Montpetit A, Cote S, Burstein E, Drouin C, Lapointe L, Boudreau M, et al. Disruption of AP1S1, causing a novel neurocutaneous syndrome, perturbs development of the skin and spinal cord. *Proc Natl Acad Sci U S A* 2009;4:1-9.
 103. Feldmeyer L, Huber M, Fellmann F, Beckmann JS, Frenk E, Hohl D. Confirmation of the origin of NISCH syndrome. *Hum Mutat* 2006;27:408-10.
 104. Hadj-Rabia S, Baala L, Vabres P, Hamel-Teillac D, Jacquemin E, Fabre M, et al. Claudin-1 gene mutations in neonatal sclerosing cholangitis associated with ichthyosis: a tight junction disease. *Gastroenterology* 2004;127:1386-90.
 105. Baala L, Hadj-Rabia S, Hamel-Teillac D, Hadchouel M, Prost C, Leal SM, et al. Homozygosity mapping of a locus for a novel

- syndromic ichthyosis to chromosome 3q27-q28. *J Invest Dermatol* 2002;119:70-6.
106. Basel-Vanagaite L, Attia R, Ishida-Yamamoto A, Rainshtein L, Ben AD, Lurie R, et al. Autosomal recessive ichthyosis with hypotrichosis caused by a mutation in ST14, encoding type II transmembrane serine protease matriptase. *Am J Hum Genet* 2007;80:467-77.
 107. Alef T, Torres S, Hausser I, Metze D, Tursen U, Lestringant GG, et al. Ichthyosis, follicular atrophoderma, and hypotrichosis caused by mutations in ST14 is associated with impaired profilaggrin processing. *J Invest Dermatol* 2009;129:862-9.
 108. Lestringant GG, Kuster W, Frossard PM, Happel R. Congenital ichthyosis, follicular atrophoderma, hypotrichosis, and hypohidrosis: a new genodermatosis? *Am J Med Genet* 1998;75:186-9.
 109. Pujol RM, Moreno A, Alomar A, De Moragas JM. Congenital ichthyosiform dermatosis with linear keratotic flexural papules and sclerosing palmoplantar keratoderma. *Arch Dermatol* 1989;125:103-6.
 110. Dahlqvist J, Klar J, Tiwari N, Schuster J, Torma H, Badhai J, et al. A single-nucleotide deletion in the POMP 5' UTR causes a transcriptional switch and altered epidermal proteasome distribution in KLICK genodermatosis. *Am J Hum Genet* 2010;86:596-603.
 111. Gottron H. Congenital angelegte symmetrische progressive erythrokeratodermie. *Zentralbl Haut Geschlechtskrankh* 1922;4:493-4.
 112. Darier MJ. Erythro-kératodermie verruqueuse en nappes, symétrique et progressive. *Bull Soc Fr Dermatol Syph* 1911;2:252-64.
 113. Mendes da Costa S. Erythro- et keratodermia variabilis in a mother and daughter. *Acta Derm Venereol* 1925;6:255-61.
 114. Richard G, Brown N, Rouan F, Van der Schroeff JG, Bijlsma E, Eichenfield LF, et al. Genetic heterogeneity in erythrokeratodermia variabilis: novel mutations in the connexin gene GJB4 (Cx30.3) and genotype-phenotype correlations. *J Invest Dermatol* 2003;120:601-9.
 115. Richard G, Smith LE, Bailey RA, Itin P, Hohl D, Epstein EH Jr, et al. Mutations in the human connexin gene GJB3 cause erythrokeratodermia variabilis. *Nat Genet* 1998;20:366-9.
 116. Macari F, Landau M, Cousin P, Mevorah B, Brenner S, Panizzon R, et al. Mutation in the gene for connexin 30.3 in a family with erythrokeratodermia variabilis. *Am J Hum Genet* 2000;67:1296-301.
 117. van Steensel MA, Oranje AP, Van der Schroeff JG, Wagner A, van Geel M. The missense mutation G12D in connexin30.3 can cause both erythrokeratodermia variabilis of Mendes da Costa and progressive symmetric erythrokeratodermia of Gottron. *Am J Med Genet A* 2009;149A:657-61.
 118. Burns FS. A case of generalized congenital keratoderma with unusual involvement of the eyes, ears, and nasal and buccous membranes. *J Cutan Dis* 1915;33:255-60.
 119. Skinner BA, Greist MC, Norins AL. The keratitis, ichthyosis, and deafness (KID) syndrome. *Arch Dermatol* 1981;117:285-9.
 120. Gulzow J, Anton-Lamprecht I. Ichthyosis hystrix gravior typus Rheydt: an otologic-dermatologic syndrome (Ichthyosis hystrix gravior Typus Rheydt: ein otologisch-dermatologisches Syndrom). *Laryngol Rhinol Otol Stuttg* 1977;56:949-55.
 121. Richard G, Rouan F, Willoughby CE, Brown N, Chung P, Ryyanen M, et al. Missense mutations in GJB2 encoding connexin-26 cause the ectodermal dysplasia keratitis-ichthyosis-deafness syndrome. *Am J Hum Genet* 2002;70:1341-8.
 122. van Steensel MA, Steijlen PM, Bladergroen RS, Hoefsloot EH, van Ravenswaaij-Arts CM, van Geel M. A phenotype resembling the Clouston syndrome with deafness is associated with a novel missense GJB2 mutation. *J Invest Dermatol* 2004;123:291-3.
 123. Netherton EW. A unique case of trichorrhexis nodosa; bamboo hairs. *AMA Arch Dermatol* 1958;78:483-7.
 124. Levy SB, Goldsmith LA. The peeling skin syndrome. *J Am Acad Dermatol* 1982;7:606-13.
 125. Chavanas S, Bodemer C, Rochat A, Hamel-Teillac D, Ali M, Irvine AD, et al. Mutations in SPINK5, encoding a serine protease inhibitor, cause Netherton syndrome. *Nat Genet* 2000;25:141-2.
 126. Komatsu N, Suga Y, Saijoh K, Liu AC, Khan S, Mizuno Y, et al. Elevated human tissue kallikrein levels in the stratum corneum and serum of peeling skin syndrome-type B patients suggests an over-desquamation of corneocytes. *J Invest Dermatol* 2006;126:2338-42.
 127. Vohwinkel KH. Keratoma hereditarium mutilans. *Arch Dermatol Syph* 1929;158:354-64.
 128. Maestrini E, Korge BP, Ocana-Sierra J, Calzolari E, Cambiaghi S, Scudder PM, et al. A missense mutation in connexin26, D66H, causes mutilating keratoderma with sensorineural deafness (Vohwinkel's syndrome) in three unrelated families. *Hum Mol Genet* 1999;8:1237-43.
 129. Stulli L. Di una variata cutanea. Lettera al direttore dell'Antologia. *Estratti dall'Antologia di Firenze* 1826;71-72:1-3.
 130. Fischer J, Bouadjar B, Heilig R, Huber M, Lefevre C, Jobard F, et al. Mutations in the gene encoding SLURP-1 in Mal de Meleda. *Hum Mol Genet* 2001;10:875-80.
 131. Papillon M, Lefèvre P. Deux cas de kératodermie palmaire et plantaire symétrique familiale (maladie de Meleda) chez le frère et la soeur. Coexistence dans les deux cas d'altérations dentaires graves. *Bull Soc Fr Dermatol Syph* 1924;31:82-7.
 132. Toomes C, James J, Wood AJ, Wu CL, McCormick D, Lench N, et al. Loss-of-function mutations in the cathepsin C gene result in periodontal disease and palmoplantar keratosis. *Nat Genet* 1999;23:421-4.
 133. Coulombe PA, Hutton ME, Letai A, Hebert A, Paller AS, Fuchs E. Point mutations in human keratin 14 genes of epidermolysis bullosa simplex patients: genetic and functional analyses. *Cell* 1991;66:1301-11.
 134. Lane EB, Rugg EL, Navsaria H, Leigh IM, Heagerty AH, Ishida-Yamamoto A, et al. A mutation in the conserved helix termination peptide of keratin 5 in hereditary skin blistering. *Nature* 1992;356:244-6.
 135. Fine JD, Eady RA, Bauer EA, Bauer JW, Bruckner-Tuderman L, Heagerty A, et al. The classification of inherited epidermolysis bullosa (EB): report of the third international consensus meeting on diagnosis and classification of EB. *J Am Acad Dermatol* 2008;58:931-50.
 136. Haenssle HA, Finkenrath A, Hausser I, Oji V, Traupe H, Hennies HC, et al. Effective treatment of severe thermodyregulation by oral retinoids in a patient with recessive congenital lamellar ichthyosis. *Clin Exp Dermatol* 2008;33:578-81.
 137. DiGiovanna JJ, Priolo M, Itin P. Approach towards a new classification for ectodermal dysplasias: integration of the clinical and molecular knowledge. *Am J Med Genet A* 2009;149A:2068-70.
 138. Salinas CF, Jorgenson RJ, Wright JT, DiGiovanna JJ, Fete MD. 2008 International conference on ectodermal dysplasias classification: conference report. *Am J Med Genet A* 2009;149A:1958-69.
 139. Plantin P, Gavanou J, Jouan N, Leroy JP, Guillet G. Collodion skin: a misdiagnosed but frequent clinical aspect of anhidrotic ectodermal dysplasia during the neonatal period (Peau collodionnée: un aspect clinique méconnu mais fréquent des

- dysplasies ectodermiques anhidrotiques en période néonatale). *Ann Dermatol Venereol* 1992;119:821-3.
140. Thomas C, Suranyi E, Pride H, Tyler W. A child with hypohidrotic ectodermal dysplasia with features of a colloid membrane. *Pediatr Dermatol* 2006;23:251-4.
141. Navarro CL, De Sandre-Giovannoli A, Bernard R, Boccaccio I, Boyer A, Genevieve D, et al. Lamin A and ZMPSTE24 (FACE-1) defects cause nuclear disorganization and identify restrictive dermopathy as a lethal neonatal laminopathy. *Hum Mol Genet* 2004;13:2493-503.
142. Lowry RB, Machin GA, Morgan K, Mayock D, Marx L. Congenital contractures, edema, hyperkeratosis, and intrauterine growth retardation: a fatal syndrome in Hutterite and Mennonite kindreds. *Am J Med Genet* 1985;22:531-43.
143. Antoine T. Ein Fall von allgemeiner, angeborener Hautatrophie. *Monatsschr Geburtsh Gynaekol* 1929;81:276-83.
144. Manning MA, Cunniff CM, Colby CE, El-Sayed YY, Hoyme HE. Neu-Laxova syndrome: detailed prenatal diagnostic and post-mortem findings and literature review. *Am J Med Genet A* 2004;125A:240-9.
145. Happle R, Koch H, Lenz W. The CHILD syndrome: congenital hemidysplasia with ichthyosiform erythroderma and limb defects. *Eur J Pediatr* 1980;134:27-33.
146. König A, Happle R, Bornholdt D, Engel H, Grzeschik KH. Mutations in the NSDHL gene, encoding a 3beta-hydroxysteroid dehydrogenase, cause CHILD syndrome. *Am J Med Genet* 2000;90:339-46.
147. Happle R, Matthiass HH, Macher E. Sex-linked chondrodysplasia punctata? *Clin Genet* 1977;11:73-6.
148. Darier J. Psorosperme folliculaire végétante. *Ann Dermatol Syph* 1889;10:597-612.
149. White J. A case of keratosis (ichthyosis) follicularis. *J Cutan Dis* 1889;7:201-9.
150. Hailey H, Hailey H. Familial benign chronic pemphigus. *Arch Dermatol Syph* 1939;39:679-85.
151. Sakuntabhai A, Ruiz-Perez V, Carter S, Jacobsen N, Burge S, Monk S, et al. Mutations in ATP2A2, encoding a Ca²⁺ pump, cause Darier disease. *Nat Genet* 1999;21:271-7.
152. Hu Z, Bonifas JM, Beech J, Bench G, Shigihara T, Ogawa H, et al. Mutations in ATP2C1, encoding a calcium pump, cause Hailey-Hailey disease. *Nat Genet* 2000;24:61-5.
153. Madison KC. Barrier function of the skin: "la raison d'être" of the epidermis. *J Invest Dermatol* 2003;121:231-41.
154. Attenborough D. Life on earth. Boston: Little Brown; 1980.
155. Blank IH. Further observations on factors which influence the water content of the stratum corneum. *J Invest Dermatol* 1953;21:259-71.
156. Winsor T, Burge GE. Differential roles of layers of human epigastric skin on diffusion rate of water. *Arch Intern Med* 1944;74:428-36.
157. Elias PM. Epidermal lipids, barrier function, and desquamation. *J Invest Dermatol* 1983;80:44-49s.
158. Williams ML. The ichthyoses—pathogenesis and prenatal diagnosis: a review of recent advances. *Pediatr Dermatol* 1983;1:1-24.
159. Demerjian M, Crumrine DA, Milstone LM, Williams ML, Elias PM. Barrier dysfunction and pathogenesis of neutral lipid storage disease with ichthyosis (Chanarin-Dorfman syndrome). *J Invest Dermatol* 2006;126:2032-8.
160. Elias PM, Schmuth M, Uchida Y, Rice RH, Behne M, Crumrine D, et al. Basis for the permeability barrier abnormality in lamellar ichthyosis. *Exp Dermatol* 2002;11:248-56.
161. Elias PM, Crumrine D, Rassner U, Hachem JP, Menon GK, Man W, et al. Basis for abnormal desquamation and permeability barrier dysfunction in RXLI. *J Invest Dermatol* 2004;122:314-9.
162. Hachem JP, Houben E, Crumrine D, Man MQ, Schurer N, Roelandt T, et al. Serine protease signaling of epidermal permeability barrier homeostasis. *J Invest Dermatol* 2006;126:2074-86.
163. Holleran WM, Ginns EI, Menon GK, Grundmann JU, Fartasch M, McKinney CE, et al. Consequences of beta-glucocerebrosidase deficiency in epidermis: ultrastructure and permeability barrier alterations in Gaucher disease. *J Clin Invest* 1994;93:1756-64.
164. Schmuth M, Fluhr JW, Crumrine DC, Uchida Y, Hachem JP, Behne M, et al. Structural and functional consequences of loricrin mutations in human loricrin keratoderma (Vohwinkel syndrome with ichthyosis). *J Invest Dermatol* 2004;122:909-22.
165. Descargues P, Deraison C, Bonnart C, Kreft M, Kishibe M, Ishida-Yamamoto A, et al. Spink5-deficient mice mimic Netherton syndrome through degradation of desmoglein 1 by epidermal protease hyperactivity. *Nat Genet* 2005;37:56-65.
166. Yanagi T, Akiyama M, Nishihara H, Sakai K, Nishie W, Tanaka S, et al. Harlequin ichthyosis model mouse reveals alveolar collapse and severe fetal skin barrier defects. *Hum Mol Genet* 2008;17:3075-83.
167. Matsuki M, Yamashita F, Ishida-Yamamoto A, Yamada K, Kinoshita C, Fushiki S, et al. Defective stratum corneum and early neonatal death in mice lacking the gene for transglutaminase 1 (keratinocyte transglutaminase). *Proc Natl Acad Sci U S A* 1998;95:1044-9.
168. Epp N, Furstenberger G, Müller K, de Juanes S, Leitges M, Hausser I, et al. 12R-lipoxygenase deficiency disrupts epidermal barrier function. *J Cell Biol* 2007;177:173-82.
169. Furuse M, Hata M, Furuse K, Yoshida Y, Haratake A, Sugitani Y, et al. Claudin-based tight junctions are crucial for the mammalian epidermal barrier: a lesson from claudin-1-deficient mice. *J Cell Biol* 2002;156:1099-111.
170. Feingold KR. The regulation of epidermal lipid synthesis by permeability barrier requirements. *Crit Rev Ther Drug Carrier Syst* 1991;8:193-210.
171. Ghadially R, Brown BE, Sequeira-Martin SM, Feingold KR, Elias PM. The aged epidermal permeability barrier: structural, functional, and lipid biochemical abnormalities in humans and a senescent murine model. *J Clin Invest* 1995;95:2281-90.
172. Williams ML, Elias PM. From basket weave to barrier: unifying concepts for the pathogenesis of the disorders of cornification. *Arch Dermatol* 1993;129:626-9.
173. Juanes SD, Epp N, Latzko S, Neumann M, Furstenberger G, Hausser I, et al. Development of an ichthyosiform phenotype in Alox12b-deficient mouse skin transplants. *J Invest Dermatol* 2009;129:1429-36.
174. Ballabio A, Parenti G, Carrozzo R, Sebastio G, Andria G, Buckle V, et al. Isolation and characterization of a steroid sulfatase cDNA clone: genomic deletions in patients with X-chromosome-linked ichthyosis. *Proc Natl Acad Sci U S A* 1987;84:4519-23.
175. Chipev CC, Korge BP, Markova N, Bale SJ, DiGiovanna JJ, Compton JG, et al. A leucine—proline mutation in the H1 subdomain of keratin 1 causes epidermolytic hyperkeratosis. *Cell* 1992;70:821-8.
176. Compton JG, DiGiovanna JJ, Santucci SK, Kearns KS, Amos CI, Abangan DL, et al. Linkage of epidermolytic hyperkeratosis to the type II keratin gene cluster on chromosome 12q. *Nat Genet* 1992;1:301-5.
177. Grzeschik KH, Bornholdt D, Oeffner F, König A, del Carmen BM, Enders H, et al. Deficiency of PORCN, a regulator of Wnt

- signaling, is associated with focal dermal hypoplasia. *Nat Genet* 2007;39:833-5.
178. Jansen GA, Ofman R, Ferdinandusse S, Ijlst L, Muijsers AO, Skjeldal OH, et al. Refsum disease is caused by mutations in the phytanoyl-CoA hydroxylase gene. *Nat Genet* 1997;17:190-3.
 179. Jansen GA, Waterham HR, Wanders RJ. Molecular basis of Refsum disease: sequence variations in phytanoyl-CoA hydroxylase (PHYH) and the PTS2 receptor (PEX7). *Hum Mutat* 2004;23:209-18.
 180. Oeffner F, Fischer G, Happle R, König A, Betz RC, Bornholdt D, et al. IFAP syndrome is caused by deficiency in MBTPS2, an intramembrane zinc metalloprotease essential for cholesterol homeostasis and ER stress response. *Am J Hum Genet* 2009;84:459-67.
 181. Rothnagel JA, Dominey AM, Dempsey LD, Longley MA, Greenhalgh DA, Gagne TA, et al. Mutations in the rod domains of keratins 1 and 10 in epidermolytic hyperkeratosis. *Science* 1992;257:1128-30.
 182. Rothnagel JA, Traupe H, Wojcik S, Huber M, Hohl D, Pittelkow MR, et al. Mutations in the rod domain of keratin 2e in patients with ichthyosis bullosa of Siemens. *Nat Genet* 1994;7:485-90.
 183. Smith FJ, Irvine AD, Terron-Kwiatkowski A, Sandilands A, Campbell LE, Zhao Y, et al. Loss-of-function mutations in the gene encoding filaggrin cause ichthyosis vulgaris. *Nat Genet* 2006;38:337-42.
 184. Stefanini M, Lagomarsini P, Gilliani S, Nardo T, Botta E, Peserico A, et al. Genetic heterogeneity of the excision repair defect associated with trichothiodystrophy. *Carcinogenesis* 1993;14:1101-5.
 185. Takayama K, Salazar EP, Broughton BC, Lehmann AR, Sarasin A, Thompson LH, et al. Defects in the DNA repair and transcription gene ERCC2(XPD) in trichothiodystrophy. *Am J Hum Genet* 1996;58:263-70.
 186. Tsuji S, Choudary PV, Martin BM, Stubblefield BK, Mayor JA, Barranger JA, et al. A mutation in the human glucocerebrosidase gene in neuronopathic Gaucher's disease. *N Engl J Med* 1987;316:570-5.
 187. Mizrachi-Koren M, Shemer S, Morgan M, Indelman M, Khamaysi Z, Petronius D, et al. Homozygosity mapping as a screening tool for the molecular diagnosis of hereditary skin diseases in consanguineous populations. *J Am Acad Dermatol* 2006;55:393-401.
 188. Lugassy J, Hennies HC, Indelman M, Khamaysi Z, Bergman R, Sprecher E. Rapid detection of homozygous mutations in congenital recessive ichthyosis. *Arch Dermatol Res* 2008;300:81-5.
 189. Roop D. Defects in the barrier. *Science* 1995;267:474-5.
 190. Bitoun E, Bodemer C, Amiel J, de Prost Y, Stoll C, Calvas P, et al. Prenatal diagnosis of a lethal form of Netherton syndrome by SPINK5 mutation analysis. *Prenat Diagn* 2002;22:121-6.
 191. Muller FB, Hausser I, Berg D, Casper C, Maiwald R, Jung A, et al. Genetic analysis of a severe case of Netherton syndrome and application for prenatal testing. *Br J Dermatol* 2002;146:495-9.
 192. Sprecher E, Chavanas S, DiGiovanna JJ, Amin S, Nielsen K, Prendiville JS, et al. The spectrum of pathogenic mutations in SPINK5 in 19 families with Netherton syndrome: implications for mutation detection and first case of prenatal diagnosis. *J Invest Dermatol* 2001;117:179-87.
 193. Rothnagel JA, Longley MA, Holder RA, Kuster W, Roop DR. Prenatal diagnosis of epidermolytic hyperkeratosis by direct gene sequencing. *J Invest Dermatol* 1994;102:13-6.
 194. Rothnagel JA, Lin MT, Longley MA, Holder RA, Hazen PG, Levy ML, et al. Prenatal diagnosis for keratin mutations to exclude transmission of epidermolytic hyperkeratosis. *Prenat Diagn* 1998;18:826-30.
 195. Tsuji-Abe Y, Akiyama M, Nakamura H, Takizawa Y, Sawamura D, Matsunaga K, et al. DNA-based prenatal exclusion of bullous congenital ichthyosiform erythroderma at the early stage, 10 to 11 weeks' of pregnancy, in two consequent siblings. *J Am Acad Dermatol* 2004;51:1008-11.
 196. Sillen A, Holmgren G, Wadelius C. First prenatal diagnosis by mutation analysis in a family with Sjögren-Larsson syndrome. *Prenat Diagn* 1997;17:1147-9.
 197. Yanagi T, Akiyama M, Sakai K, Nagasaki A, Ozawa N, Kosaki R, et al. DNA-based prenatal exclusion of harlequin ichthyosis. *J Am Acad Dermatol* 2008;58:653-6.
 198. Akiyama M, Titeux M, Sakai K, McMillan JR, Tonasso L, Calvas P, et al. DNA-based prenatal diagnosis of harlequin ichthyosis and characterization of ABCA12 mutation consequences. *J Invest Dermatol* 2007;127:568-73.
 199. Anton-Lamprecht I. The skin. In: Papdimitriou JM, Henderon DW, Sagnolo DV, editors. *Diagnostic ultrastructure of non-neoplastic diseases: diagnostic ultrastructure of non-neoplastic diseases*. Edinburgh: Churchill-Livingstone; 1992. pp. 459-550.
 200. Anton-Lamprecht I. Ultrastructural identification of basic abnormalities as clues to genetic disorders of the epidermis. *J Invest Dermatol* 1994;103:6-125.
 201. Anton-Lamprecht I, Schnyder UW. Ultrastructural distinction of autosomal dominant ichthyosis vulgaris and X-linked recessive ichthyosis. *Clin Genet* 1976;10:245-7.
 202. Oji V, Sella N, Sandilands A, Gruber R, Gerss J, Huffmeier U, et al. Ichthyosis vulgaris: novel FLG mutations in the German population and high presence of CD1a⁺ cells in the epidermis of the atopic subgroup. *Br J Dermatol* 2009;160:771-81.
 203. Dale BA, Holbrook KA, Fleckman P, Kimball JR, Brumbaugh S, Sybert VP. Heterogeneity in harlequin ichthyosis, an inborn error of epidermal keratinization: variable morphology and structural protein expression and a defect in lamellar granules. *J Invest Dermatol* 1990;94:6-18.
 204. Akiyama M, Sakai K, Sato T, McMillan JR, Goto M, Sawamura D, et al. Compound heterozygous ABCA12 mutations including a novel nonsense mutation underlie harlequin ichthyosis. *Dermatology* 2007;215:155-9.
 205. Ishida-Yamamoto A. Loricrin keratoderma: a novel disease entity characterized by nuclear accumulation of mutant loricrin. *J Dermatol Sci* 2003;31:3-8.
 206. Arnold ML, Anton-Lamprecht I, Melz-Rothfuss B, Hartschuh W. Ichthyosis congenita type III: clinical and ultrastructural characteristics and distinction within the heterogeneous ichthyosis congenita group. *Arch Dermatol Res* 1988;280:268-78.
 207. Brusasco A, Gelmetti C, Tadini G, Caputo R. Ichthyosis congenita type IV: a new case resembling diffuse cutaneous mastocytosis. *Br J Dermatol* 1997;136:377-9.
 208. Niemi KM, Kanerva L, Kuokkanen K. Recessive ichthyosis congenita type II. *Arch Dermatol Res* 1991;283:211-8.
 209. Niemi KM, Kuokkanen K, Kanerva L, Ignatius J. Recessive ichthyosis congenita type IV. *Am J Dermatopathol* 1993;15:224-8.
 210. Niemi KM, Kanerva L, Kuokkanen K, Ignatius J. Clinical, light and electron microscopic features of recessive congenital ichthyosis type I. *Br J Dermatol* 1994;130:626-33.
 211. Pigg M, Gedde-Dahl T Jr, Cox D, Hausser I, Anton-Lamprecht I, Dahl N. Strong founder effect for a transglutaminase 1 gene mutation in lamellar ichthyosis and congenital ichthyosiform erythroderma from Norway. *Eur J Hum Genet* 1998;6:589-96.
 212. Descargues P, Deraison C, Prost C, Freitag S, Mazereeuw-Hautier J, D'Alessio M, et al. Corneodesmosomal cadherins

- are preferential targets of stratum corneum trypsin- and chymotrypsin-like hyperactivity in Netherton syndrome. *J Invest Dermatol* 2006;126:1622-32.
213. Wells RS, Kerr CB. The histology of ichthyosis. *J Invest Dermatol* 1966;46:530-5.
214. Fleckman P, Brumbaugh S. Absence of the granular layer and keratohyalin define a morphologically distinct subset of individuals with ichthyosis vulgaris. *Exp Dermatol* 2002;11:327-36.
215. Bergman R, Khamaysi Z, Sprecher E. A unique pattern of dyskeratosis characterizes epidermolytic hyperkeratosis and epidermolytic palmoplantar keratoderma. *Am J Dermatopathol* 2008;30:101-5.
216. Ross R, DiGiovanna JJ, Capaldi L, Argenyi Z, Fleckman P, Robinson-Bostom L. Histopathologic characterization of epidermolytic hyperkeratosis: a systematic review of histology from the national registry for ichthyosis and related skin disorders. *J Am Acad Dermatol* 2008;59:86-90.
217. Sperling LC, DiGiovanna JJ. Curly wood and tiger tails: an explanation for light and dark banding with polarization in trichothiodystrophy. *Arch Dermatol* 2003;139:1189-92.
218. Tay CH. Ichthyosiform erythroderma, hair shaft abnormalities, and mental and growth retardation: a new recessive disorder. *Arch Dermatol* 1971;104:4-13.
219. Schlucker S, Liang C, Strehle KR, DiGiovanna JJ, Kraemer KH, Levin IW. Conformational differences in protein disulfide linkages between normal hair and hair from subjects with trichothiodystrophy: a quantitative analysis by Raman microspectroscopy. *Biopolymers* 2006;82:615-22.
220. Liang C, Morris A, Schlucker S, Imoto K, Price VH, Menefee E, et al. Structural and molecular hair abnormalities in trichothiodystrophy. *J Invest Dermatol* 2006;126:2210-6.
221. Gruber R, Janecke AR, Fauth C, Utermann G, Fritsch PO, Schmuth M. Filaggrin mutations p.R501X and c.2282del4 in ichthyosis vulgaris. *Eur J Hum Genet* 2007;15:179-84.
222. Bitoun E, Micheloni A, Lamant L, Bonnart C, Tartaglia-Polcini A, Cobbold C, et al. LEKTI proteolytic processing in human primary keratinocytes, tissue distribution and defective expression in Netherton syndrome. *Hum Mol Genet* 2003;12:2417-30.
223. Ong C, O'Toole EA, Ghali L, Malone M, Smith VV, Callard R, et al. LEKTI demonstrable by immunohistochemistry of the skin: a potential diagnostic skin test for Netherton syndrome. *Br J Dermatol* 2004;151:1253-7.
224. Raghunath M, Tontsidou L, Oji V, Aufenvenne K, Schurmeyer-Horst F, Jayakumar A, et al. SPINK5 and Netherton syndrome: novel mutations, demonstration of missing LEKTI, and differential expression of transglutaminases. *J Invest Dermatol* 2004;123:474-83.
225. Raghunath M, Hennies HC, Velten F, Wiebe V, Steinert PM, Reis A, et al. A novel in situ method for the detection of deficient transglutaminase activity in the skin. *Arch Dermatol Res* 1998;290:621-7.
226. Hohl D, Aeschlimann D, Huber M. In vitro and rapid in situ transglutaminase assays for congenital ichthyoses—a comparative study. *J Invest Dermatol* 1998;110:268-71.
227. Jeon S, Djian P, Green H. Inability of keratinocytes lacking their specific transglutaminase to form cross-linked envelopes: absence of envelopes as a simple diagnostic test for lamellar ichthyosis. *Proc Natl Acad Sci U S A* 1998;95:687-90.
228. Has C, Seedorf U, Kannenberg F, Bruckner-Tuderman L, Folkers E, Folster-Holst R, et al. Gas chromatography-mass spectrometry and molecular genetic studies in families with the Conradi-Hünemann-Happle syndrome. *J Invest Dermatol* 2002;118:851-8.
229. Traupe H, Burgdorf WHC. Treatment of ichthyosis—there is always something you can do! In Memoriam: Wolfgang Küster. *J Am Acad Dermatol* 2007;57:542-7.
230. Shwayder T. Disorders of keratinization: diagnosis and management. *Am J Clin Dermatol* 2004;5:17-29.
231. Kuster W. Ichthyoses: suggestions for an improved therapy. *Dtsch Arztebl* 2006;103:1484-9.
232. Oji V, Traupe H. Ichthyosis: clinical manifestations and practical treatment options. *Am J Clin Dermatol* 2009;10:351-64.
233. Yamamura S, Kinoshita Y, Kitamura N, Kawai S, Kobayashi Y. Neonatal salicylate poisoning during the treatment of a collodion baby. *Clin Pediatr* 2002;41:451-2.
234. Moskowitz DG, Fowler AJ, Heyman MB, Cohen SP, Crumrine D, Elias PM, et al. Pathophysiologic basis for growth failure in children with ichthyosis: an evaluation of cutaneous ultrastructure, epidermal permeability barrier function, and energy expenditure. *J Pediatr* 2004;145:82-92.
235. Fowler AJ, Moskowitz DG, Wong A, Cohen SP, Williams ML, Heyman MB. Nutritional status and gastrointestinal structure and function in children with ichthyosis and growth failure. *J Pediatr Gastroenterol Nutr* 2004;38:164-9.

Partially disturbed lamellar granule secretion in mild congenital ichthyosiform erythroderma with *ALOX12B* mutations

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Conflicts of interest

None declared.

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Congenital ichthyosiform erythroderma (CIE) (OMIM 242100) is a major type of autosomal recessive congenital ichthyosis (ARCI) showing generalized scaling and erythroderma without blister formation.¹ Mutations in *ALOX12B* (OMIM 603741), encoding 12R-lipoxygenase (LOX), were identified in patients with CIE in 2002.² To date, several *ALOX12B* mutations have been reported in CIE families.^{3,4} LOXs are a family of nonhaem, iron-containing dioxygenases which catalyse dioxygenation of fatty acids with one or more (Z,Z)-1,4-pentadiene moieties.⁵ Three members of the human LOX family, 15-LOX-2, 12R-LOX and eLOX-3, are preferentially expressed in the skin.^{5,6} The 12R-LOX pathway leads to hepxilin B3 and trioxilin B3⁷ resulting in 20-carboxy-trioxilin A3,⁵ which is thought to be a key biological regulator in the skin.⁸ 12R-LOX deficiency results in a CIE phenotype in humans^{2,9,10} and in mice.^{11,12} We report that a Japanese patient with CIE, harbouring one previously unreported *ALOX12B* mutation p.Arg442Gln and another known mutation p.Arg432X, showed partially disturbed secretion of lamellar granule (LG) contents in the epidermis.

Case and methods

The patient was the first child of healthy, unrelated Japanese parents. There was no family history of any related disorders. The male child was born via uncomplicated, vaginal full-term delivery. The newborn was covered by a collodion membrane and showed thick scales on a background of erythroderma over his entire body, with skin fissures on the trunk (Fig. 1a–c). Moderate ectropion and eclabium were seen. Hands and feet were oedematous, and the palms and soles were involved. The patient was treated with a topical application of white petrolatum with an occlusive dressing technique in a humid incubator. The hyperkeratosis and oedema were remarkably reduced within 2 weeks. At age 3.5 months, the patient showed mild white to grey-coloured scales over the erythematous skin covering his entire body (Fig. 1d–f).

Mutation analysis of *ALOX12B* was performed using genomic DNA isolated from peripheral blood cells of the patient and his parents.

Results and discussion

Mutation analysis of *ALOX12B* revealed that the patient was a compound heterozygote for a known nonsense mutation p.Arg432X (c.1294C>T) in exon 10 and a previously unreported missense mutation p.Arg442Gln (c.1325C>T) in exon

10 (GenBank NM_001139.2) (Fig. 1g, h). The nonsense mutation p.Arg432X was present in a heterozygous fashion in his mother, although p.Arg442Gln was not found in the parents and was thought to be a *de novo* mutation. The mutations were verified by mutant allele-specific amplification analysis. No mutation was found in the sequence analysis of 200 alleles from 100 normal, unrelated Japanese individuals, and therefore it is unlikely to be a polymorphism (data not shown). No other pathogenic mutations were found in *TGM1* (OMIM 190195), *ABCA12* (OMIM 607800), *NIPAL4* (ichthyin; OMIM 609383), *CYP4F22* (previously known as *FLJ39501*; OMIM 611495) or *ALOXE3* (OMIM 607206) by direct sequencing analysis.

p.Arg432X results in a serious truncation of the 12R-LOX peptide, losing approximately half of the C-terminal catalytic LOX domain, and is thought to have a serious effect on the enzyme activity.

The arginine residue mutated by p.Arg442Gln is in the central part of the C-terminal catalytic LOX domain and is highly conserved among diverse species (Fig. 1i) and human LOX family members (Fig. 1j). These facts suggest that this arginine residue might be essential for enzyme activity and that the present missense mutation affects 12R-LOX activity.

Electron microscopy of a skin biopsy specimen from the trunk at age 6 days using ruthenium tetroxide postfixation revealed irregular-sized lipid droplets in the stratum corneum.

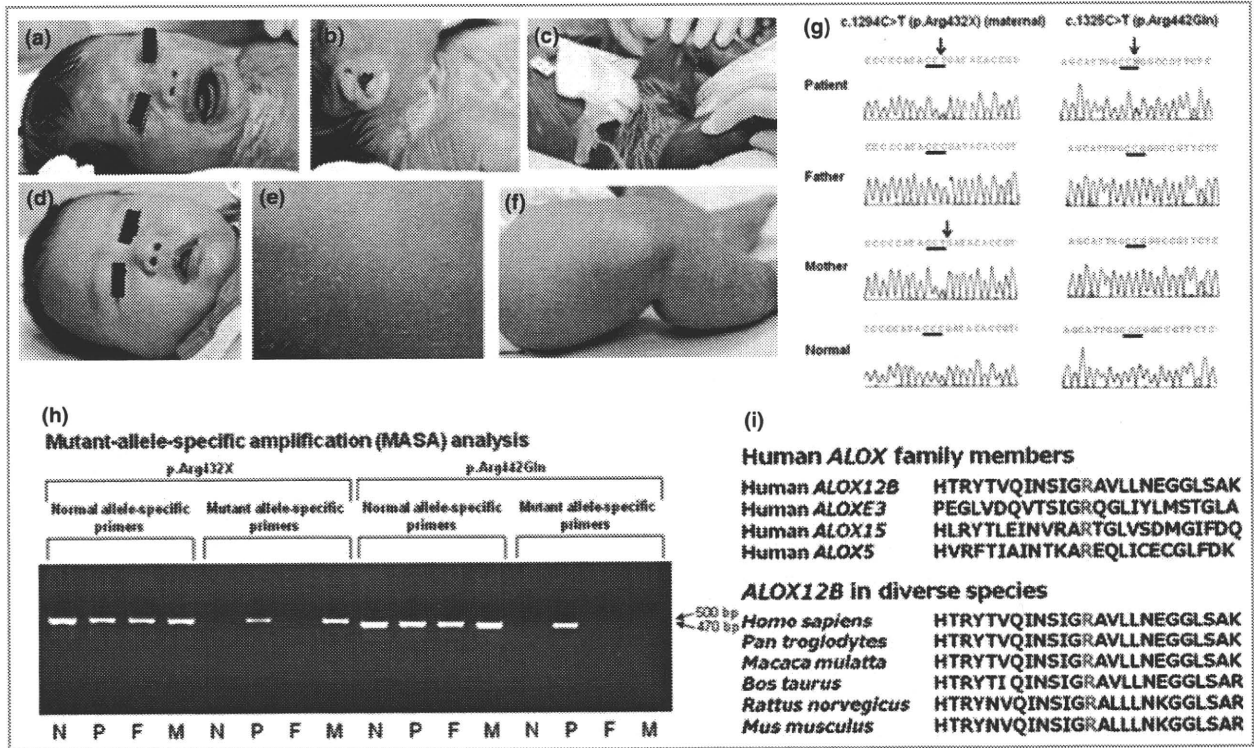


Fig 1. The patient's clinical features and compound heterozygous mutations in ALOX12B. (a–c) At 1 day of age: moderate ectropion and eclabium (a), a malformed auricle (b), and thick plate-like scales and fissures on the neck and the chest (b) and on the lower abdomen to the thigh (c). (d–f) At age 3–5 months: only slight, fine scales on the face (d), the back (e) and the thigh (f). (g) Compound heterozygous ALOX12B mutations, maternal p.Arg432X (c.1294C>T) and a novel missense mutation p.Arg442Gln (c.1325C>T) in exon 10 of the patient's genomic DNA. (h) Mutant allele-specific amplification (MASA) analysis showed a 500-bp band from p.Arg432X and a 470-bp band from p.Arg442Gln. N, normal; P, patient; F, father; M, mother. (i) Arginine 442 in 12R-lipoxygenase (LOX) altered by p.Arg442Gln; red character (R) is conserved among human ALOX family members (top) and diverse species (bottom).

Intact LGs were observed and intercellular lipid lamellae were seen in the intercellular space in the stratum corneum, although secretion of LG contents was disturbed in part in the granular layer cells (Fig. 2a–e).

Immunofluorescence staining revealed that one of the major basal keratins (keratin 5), differentiation-specific keratin (keratin 10) and ABCA12 were distributed normally in the basal layer, in the suprabasal layers, and in the granular layers, respectively, of the patient's epidermis (Fig. 2f, h, j). Immunoreactivity for cornified cell envelope-associated proteins involucrin, loricrin and transglutaminase 1 was seen normally distributed in the patient's upper epidermis (Fig. 2l, n, p).

Keratinocyte differentiation (keratinization)-specific molecules examined in the present study were normally distributed in the patient's epidermis. Also, keratinocyte differentiation defects were not morphologically or biochemically detected in *Alox12b*-disrupted mice.¹¹ These facts suggest that keratinocyte differentiation defects might not be involved in the pathogenesis of ichthyosis in CIE with ALOX12B mutations.

One report on ultrastructural features of the 12R-LOX-deficient human epidermis demonstrated lipid droplets in the cornified layers.¹³ From the study of *Alox12b* mutant mouse

skin transplants, it was suggested that 12R-LOX deficiency might affect the processing of LGs.¹⁴ In *Alox12b*-disrupted mouse skin, irregular-sized vesicles were observed in the granular layers, and the stacks of lipid lamellae representing extruded content of LGs were seen in the transition zone between the uppermost granular and the first cornified cells.¹¹ In the present study, we clearly demonstrated lipid droplets in the cornified cell layers, and partially disturbed LG content secretion into the intercellular space in the patient's epidermis. Our results suggest that partially disturbed secretion of LG contents is involved in the pathogenesis of CIE. Several ichthyosiform disorders have been shown to involve the abnormal function of LG.^{11,15} Lipid contents secreted from LG are known to form the intercellular lipid layers in the stratum corneum.¹⁶ The intercellular lipid layers are essential for the epidermal barrier function and the defective intercellular lipid layers caused by disturbed secretion of LG lipid contents are expected to result in skin barrier impairment.¹⁷ Defective skin barrier function leads to compensatory mechanisms involving epidermal hyperproliferation and hyperkeratosis, which are

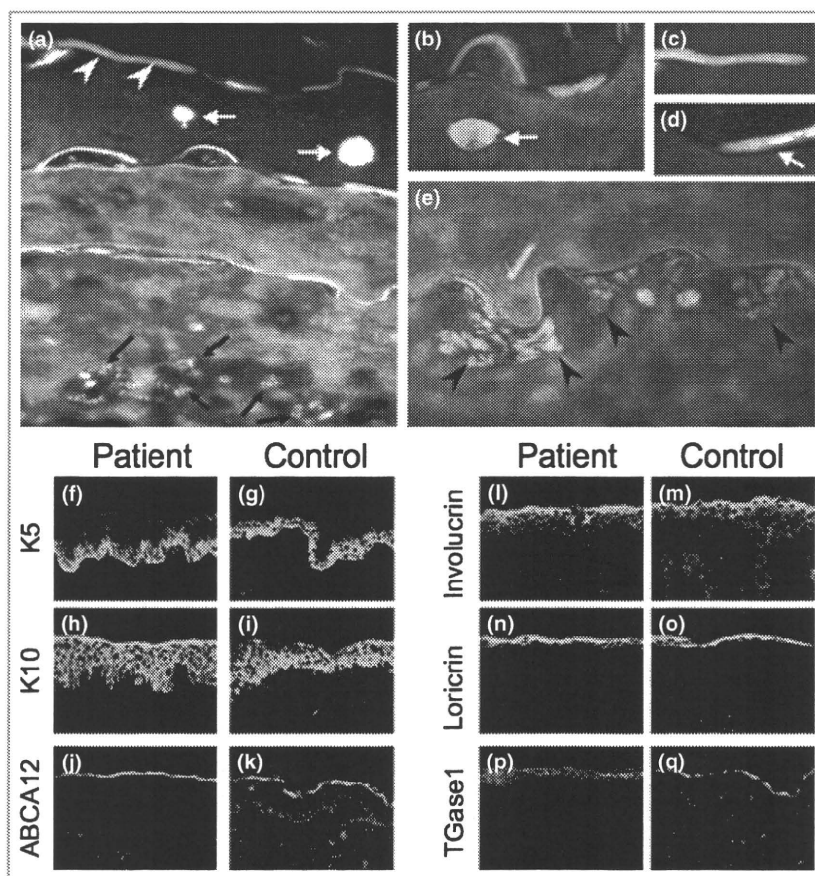


Fig 2. Disturbed secretion of lamellar granule (LG) contents from the granular layer cells and normal distribution of keratinization markers in the patient's epidermis. (a–e) Ultrastructural features of the skin biopsy specimen in the neonatal period. (a) Overall keratinization processes appeared normal. Black arrows, LGs; white arrowheads, intercellular lipid lamellae; white arrows, various-sized cytoplasmic lipid vacuoles. (b) An irregular-sized lipid vacuole (white arrow) containing the lamellar structure. (c) Intact intercellular lipid lamellae. (d) Normal cornified cell envelope (white arrow). (e) Partially congested LG secretion (arrowheads) in a granular layer cell. (f–q) Immunofluorescence staining (FITC) revealed that keratin 5, keratin 10 and ABCA12 were distributed normally in the basal layer, suprabasal layers and granular layers, respectively. For keratin 5 immunostaining, we used an antibody which cross-reacts with keratin 8. However, the labelling of this antibody is thought to reflect keratin 5 expression in this study, because keratin 8 expression is usually restricted to the simple epithelia. Cornified cell envelope-associated proteins, involucrin, loricrin and transglutaminase 1, were seen normally distributed in the upper epidermis. (f–q) Nuclear stain, red (propidium iodide). Original magnification: (a) $\times 6000$, (b) $\times 12\,000$, (c, d) $\times 18\,000$, (e) $\times 10\,000$, (f–q) $\times 20$.

observed frequently in ichthyosiform diseases.¹⁶ In this context, we can hypothesize that the partially disturbed secretion of LG contents might cause defective intercellular lipid layers in the stratum corneum, resulting in skin barrier defects and subsequent compensatory hyperkeratosis in CIE.

Patients with CIE harbouring *ALOX12B* mutations have previously been reported in African and European populations.^{3,4} Our previous studies failed to identify *ALOX12B* mutations in Japanese patients with ARCI¹⁸ and demonstrated that the frequency of *ALOX12B* mutations is expected to be low in Japanese patients with ARCI. As far as we know, the present case is the first patient with CIE with detected *ALOX12B* mutations in the Asian area and our results also confirm *ALOX12B* as one of the CIE causative genes in the Asian population. Further accumulation of CIE cases is needed to clarify the frequency of *ALOX12B* mutations in patients with CIE in Asian countries.

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References

- 1 Akiyama M, Shimizu H. An update on molecular aspects of the non-syndromic ichthyoses. *Exp Dermatol* 2008; **42**:83–9.
- 2 Jobard F, Lefèvre C, Karaduman A *et al.* Lipoxygenase-3 (*ALOXE3*) and 12(R)-lipoxygenase (*ALOX12B*) are mutated in non-bullous

- congenital ichthyosiform erythroderma (NCIE) linked to chromosome 17p13.1. *Hum Mol Genet* 2002; **11**:107–13.
- 3 Eckl KM, de Juanes S, Kurtenbach J *et al.* Molecular analysis of 250 patients with autosomal recessive congenital ichthyosis: evidence for mutation hotspots in *ALOXE3* and allelic heterogeneity in *ALOX12B*. *J Invest Dermatol* 2009; **129**:1421–8.
 - 4 Fischer J. Autosomal recessive congenital ichthyosis. *J Invest Dermatol* 2009; **129**:1319–21.
 - 5 Brash AR, Yu Z, Boeglin WE, Schneider C. The hepoxilin connection in the epidermis. *FEBS J* 2007; **274**:3494–502.
 - 6 Krieg P, Heidt M, Siebert M *et al.* Epidermis-type lipoxygenases. *Adv Exp Med Biol* 2002; **507**:165–70.
 - 7 Anton R, Camacho M, Puig L, Vila L. Hepoxilin B3 and its enzymatically formed derivative trioxilin B3 are incorporated into phospholipids in psoriatic lesions. *J Invest Dermatol* 2002; **118**:139–46.
 - 8 Lefèvre C, Bouadjar B, Ferrand V *et al.* Mutations in a new cytochrome P450 gene in lamellar ichthyosis type 3. *Hum Mol Genet* 2006; **15**:767–76.
 - 9 Eckl KM, Krieg P, Küster W *et al.* Mutation spectrum and functional analysis of epidermis-type lipoxygenases in patients with autosomal recessive congenital ichthyosis. *Hum Mutat* 2005; **26**:351–61.
 - 10 Yu Z, Schneider C, Boeglin WE, Brash AR. Mutations associated with a congenital form of ichthyosis (NCIE) inactivate the epidermal lipoxygenases 12R-LOX and eLOX3. *Biochim Biophys Acta* 2005; **3**:238–47.
 - 11 Epp N, Fürstenberger G, Müller K *et al.* 12R-lipoxygenase deficiency disrupts epidermal barrier function. *J Cell Biol* 2007; **177**:173–82.
 - 12 Moran J, Qiu H, Turbe-Doan A *et al.* A mouse mutation in the 12R-lipoxygenase, *Alox12b*, disrupts formation of the epidermal permeability barrier. *J Invest Dermatol* 2007; **127**:1893–7.
 - 13 Harting M, Brunetti-Pierri N, Chan CS *et al.* Self-healing collodion membrane and mild nonbullous congenital ichthyosiform erythroderma due to 2 novel mutations in the *ALOX12B* gene. *Arch Dermatol* 2008; **144**:351–6.
 - 14 de Juanes S, Epp N, Latzko S *et al.* Development of an ichthyosiform phenotype in *Alox12b*-deficient mouse skin transplants. *J Invest Dermatol* 2009; **129**:1429–36.
 - 15 Hershkovitz D, Mandel H, Ishida-Yamamoto A *et al.* Defective lamellar granule secretion in arthrogyrosis, renal dysfunction, and cholestasis syndrome caused by a mutation in *VPS33B*. *Arch Dermatol* 2008; **144**:334–40.
 - 16 Elias PM. Stratum corneum defensive functions: an integrated view. *J Invest Dermatol* 2005; **125**:183–200.
 - 17 Yanagi T, Akiyama M, Nishihara H *et al.* Harlequin ichthyosis model mouse reveals alveolar collapse and severe fetal skin barrier defects. *Hum Mol Genet* 2008; **17**:3075–83.
 - 18 Sakai K, Akiyama M, Yanagi T *et al.* *ABCA12* is a major causative gene for non-bullous congenital ichthyosiform erythroderma. *J Invest Dermatol* 2009; **129**:2306–9.

Prevalent *LIPH* Founder Mutations Lead to Loss of P2Y5 Activation Ability of PA-PLA₁α in Autosomal Recessive Hypotrichosis

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ABSTRACT: Autosomal recessive hypotrichosis (ARH) is characterized by sparse hair on the scalp without other abnormalities. Three genes, *DSG4*, *LIPH*, and *LPAR6* (*P2RY5*), have been reported to underlie ARH. We performed a mutation search for the three candidate genes in five independent Japanese ARH families and identified two *LIPH* mutations: c.736T>A (p.Cys246Ser) in all five families, and c.742C>A (p.His248Asn) in four of the five families. Out of 200 unrelated control alleles, we detected c.736T>A in three alleles and c.742C>A in one allele. Haplotype analysis revealed each of the two mutant alleles is derived from a respective founder. These results suggest the *LIPH* mutations are prevalent founder mutations for ARH in the Japanese population. *LIPH* encodes PA-PLA₁α (*LIPH*), a membrane-associated phosphatidic acid-preferring phospholipase A₁α. Two residues, altered by these mutations, are conserved among PA-PLA₁α of diverse species. Cys²⁴⁶ forms intramolecular disulfide bonds on the lid domain, a crucial structure for substrate recognition, and His²⁴⁸ is one amino acid of the catalytic triad. Both p.Cys246Ser- and p.His248Asn-PA-PLA₁α mutants showed complete abolition of hydrolytic activity and had no P2Y5 activation ability. These results suggest defective activation of P2Y5 due to reduced 2-acyl lysophosphatidic acid production by the mutant PA-PLA₁α is involved in the pathogenesis of ARH.

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KEY WORDS: *LIPH*; Lysophosphatidic Acid; Phosphatidic Acid; Lid Domain; Catalytic Triad; LAH2; LAH

Introduction

Autosomal recessive hypotrichosis (ARH; MIM#s 607892, 607903, 611452) is a rare form of alopecia characterized by sparse

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hair on the scalp, sparse to absent eyebrows and eyelashes, and sparse axillary and body hair. Wali et al. [2007] noted clinical similarities among three genetically distinct forms of hypotrichosis, localized autosomal recessive hypotrichosis (LAH), and proposed that the forms mapped to chromosome 18q12.1, 3q27.2, and 13q14.11–q21.32 are designated as LAH1, LAH2, and LAH3, respectively. Recently, causative genes for all three forms were identified. Mutations in the desmoglein-4 gene (*DSG4*; MIM# 607892) lead to LAH1 [Kljuic et al., 2003; Rafique et al., 2003]. Mutations in *LIPH* (MIM# 607365), which encodes membrane-associated phosphatidic acid-preferring phospholipase A₁α (PA-PLA₁α [*LIPH*]), underlie LAH2 [Ali et al., 2007; Kazantseva et al., 2006]. Most recently, Pasternack et al. [2008] and Shimomura et al. [2008] reported that mutations in the lysophosphatidic acid receptor 6 gene *LPAR6* (*P2RY5*; MIM# 609239) caused LAH3.

In this study, we searched for mutations in the *DSG4*, *LIPH*, and *LPAR6* genes in five unrelated Japanese families with ARH. Surprisingly, we found two prevalent missense mutations in the *LIPH* gene in all of the families. Furthermore, one mutation c.736T>A (p.Cys246Ser) was found in all five families, and the other mutation c.742C>A (p.His248Asn) was detected in four of the five families. We clarified that these two mutations are strong founder mutations in *LIPH* in the Japanese population. In addition, we evaluated the enzyme activity of mutant PA-PLA₁α derived from the two mutant alleles. We also analyzed the abilities of the mutant PA-PLA₁α to activate lysophosphatidic acid receptor 6 (P2Y5), to clarify the pathogenetic pathway of ARH.

Materials and Methods

Subjects

Five unrelated nonconsanguineous Japanese families A, B, C, D, and E (Fig. 1) with ARH were seen in our hospital or referred to us for the past 5 years. Families A, C, and D were from Hokkaido, the northern most major island of Japan. Families B and E were from western and central Japan, respectively. The medical ethics committee of Hokkaido University approved all the described studies. The study was conducted according to the Declaration of Helsinki Principles. The patients gave written informed consent.

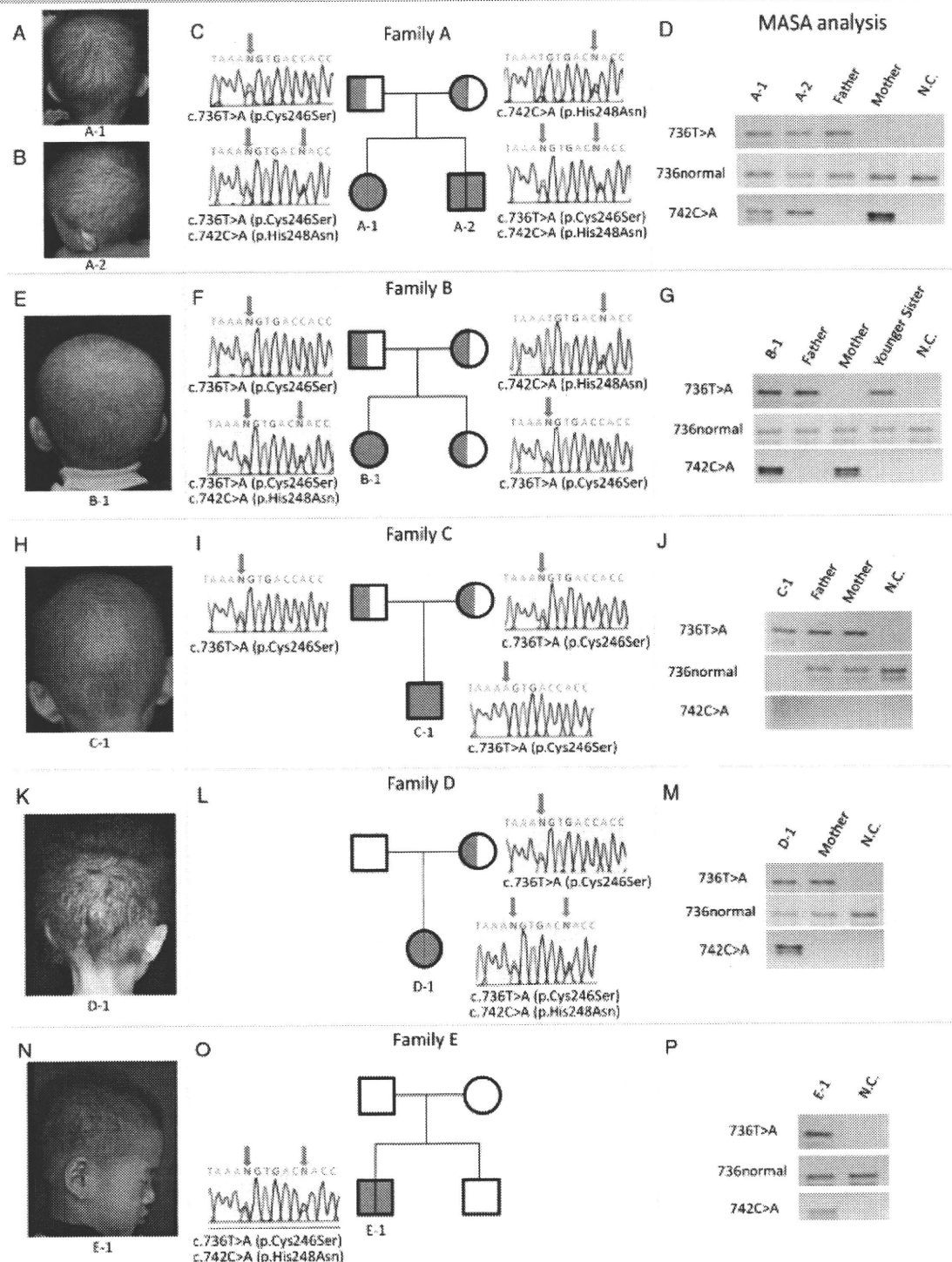


Figure 1. Clinical features of five Japanese families with ARH and identification of mutations in the *LIPH* gene. **A, B, E, H, K, N:** All the affected individuals have features of ARH, which is characterized by sparse hair on the scalp and slightly sparse to absent eyebrows and eyelashes. **C, F, I, L, O:** Pedigrees of the families. Family A (C), Family B (F), Family C (I), Family D (L), and Family E (O) are consistent with autosomal recessive inheritance. Direct sequencing of the *LIPH* gene revealed that patients A-1, A-2, B-1, D-1, and E-1 had compound heterozygous missense mutations involving c.736T>A and c.742C>A, whereas patient C-1 had a homozygous c.736T>A missense mutation. **D, G, J, M, P:** Mutant-allele-specific amplification (MASA) analysis. (Upper) With c.736T>A mutant allele-specific primers, the amplification bands from the c.736T>A mutant alleles are detected by direct sequencing as 301 bp fragments only in the patients and their family members who had the c.736T>A missense mutation, confirming the presence of the mutation. (Middle) With c.736 wild-type allele-specific primers, no PCR product was detected in patient C-1, who was homozygous for c.736T>A. PCR products from the other patients who were compound heterozygous for the two missense mutations c.736T>A and c.742C>A, from unaffected family members and from the normal control (N.C.) were amplified by wild-type allele-specific amplification. (Lower) With c.742C>A mutant-allele-specific primers, the amplification bands from the c.742C>A mutant alleles were detected as 297 bp fragments only in the PCR products from the DNA samples of the patients and their family members who had the c.742C>A missense mutation, confirming the presence of the mutation.

Mutation Detection

DSG4, *LIPH*, and *LPAR6* mutation search was performed as previously reported [Moss et al., 2004; Pasternack et al., 2008; Shimomura et al., 2008, 2009b]. Briefly, genomic DNA (gDNA) isolated from peripheral blood was subjected to polymerase chain reaction (PCR) amplification, followed by direct automated sequencing using an ABI PRISM 3100 genetic analyzer (Advanced Biotechnologies, Columbia, MD), and verification of the mutations by mutant-allele-specific amplification (MASA) analysis.

Oligonucleotide primers were designed using the Website program (www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi). The entire coding regions of *DSG4*, *LIPH*, and *LPAR6*, including the exon/intron boundaries, were sequenced using gDNA samples from patients and their family members, after fully informed consent. For normal controls, 100 healthy unrelated Japanese individuals (200 normal alleles) were studied.

The complementary DNA (cDNA) nucleotides and the amino acids of the protein were numbered based on the previous sequence information (GenBank accession number, *DSG4*; AY177664.1, *LIPH*; AY093498.1, *LPAR6*; AF000546.1) [Jin et al., 2002; Whittock and Bower, 2003]. Nucleotide numbering reflects cDNA numbering with +1 corresponding to the A of the ATG translation initiation codon in the reference sequence, according to journal guidelines (www.hgvs.org/mutnomen). The initiation codon is codon 1.

Mutant Allele-Specific Amplification Analysis

For verification of the mutation, using PCR products as a template, mutant allele specific amplification analysis was performed with mutant allele-specific primers carrying the substitution of a base at the 3'-end [Hasegawa et al., 1995; Xu et al., 2003], as follows: c.736T>A mutant allele-specific forward primer, 5'-CCAAGGATTTTCAGTATTTTAAAA-3'; c.736 normal allele-specific forward primer, 5'-CCAAGGATTTTCAGTATTTTAAAT-3'; c.742C>A mutant allele-specific forward primer, 5'-GGATTTTCAGTATTTTAAATGTGACA-3'; reverse primer, 5'-GTGCCAGCAGAAAAACAAG-3'.

PCR conditions were as follows: 94°C for 5 min, followed by 35 cycles at 94°C for 1 min, 60°C (for c.736T>A mutant amplification) or 64°C (for c.742C>A mutant amplification) for 1 min, and extension at 72°C for 7 min. Only 301- and 297-bp fragments derived from the mutant alleles were amplified with these primers and the PCR condition, respectively.

Haplotype Analysis

To determine whether the mutations c.736T>A and c.742C>A are founder mutations, we performed haplotype analysis. We constructed linkage disequilibrium (LD) blocks containing the *LIPH* gene using genotype data from the HapMap database (International HapMap Consortium, 2005). The haplotype structure with its tag-single nucleotide polymorphisms (SNPs) was determined using Haploview [Barrett et al., 2005]. We genotyped 10 tag-SNPs using the ABI PRISM 3100 genetic analyzer (Advanced Biotechnologies). Oligonucleotide primers were designed using the website program (www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi).

Construction of Mutated *LIPH* Gene Expression Vectors

Normal human full-length *LIPH* cDNA was amplified by reverse transcription-PCR using human colon-derived total RNA

[Sonoda et al., 2002]. The DNA fragment covering the coding region of PA-PLA₁α (EcoRI–EcoRI fragment) was subcloned into the EcoRI site of pCAGGS mammalian expression vector (kindly donated by Dr. Junichi Miyazaki, Osaka University) [Hiramatsu et al., 2003]. Short *LIPH* fragments (64 bp) (c.695–758) including either the c.736T>A or the c.742C>A mutation were synthesized by IDT Inc. (Coralville, IA). pCAGGS vector including the rest of the *LIPH* gene was amplified with specific primers as follows: forward (5'-CCTGTACCTGTCTTCCCTGAG-3') and reverse (5'-CAGGTTGATCCAATCCTCCA-3'). PCR was carried out using KOD-Plus-Ver.2 (Toyobo, Osaka, Japan) according to the instructions. Finally, the synthesized mutated DNA fragments were ligated with the amplified pCAGGS vector including the *LIPH* gene without 64 bp oligonucleotide (c.695–758) using a Ligation-Convenience Kit (Nippon Gene Co., Tokyo, Japan).

Expression of Mutated PA-PLA₁α in HEK293 Cells

To investigate the molecular defects underlying the mutations that were identified in this study, we synthesized p.Cys246Ser or p.His248Asn mutations in PA-PLA₁α expression constructs and compared mutant protein expression with wild-type (WT) and p.Ser154Ala PA-PLA₁α protein. Previously, Sonoda et al. [2002] reported that Ser¹⁵⁴ was the active catalytic residue and that the p.Ser154Ala mutant PA-PLA₁α had complete loss of enzyme activity, although the amount of p.Ser154Ala mutant protein expressed was almost the same as that of WT protein. Thus, we used the p.Ser154Ala mutant as a loss-of-function mutant control in this study.

HEK293 cells were maintained in Dulbecco's modified Eagle's medium supplemented with antibiotics and 10% fetal bovine serum under an atmosphere of 5% CO₂ at 37°C. The resulting cDNAs were used to transfect HEK293 cells using LipofectAMINE 2000 reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. HEK293 cells were transfected with WT, p.Ser154Ala (control loss-of-function mutant) [Sonoda et al., 2002], p.Cys246Ser or p.His248Asn PA-PLA₁α.

Preparation of Cell Supernatants and Lysates and Western Blotting

HEK293 cells transfected with pCAGGS vector were maintained for an additional 24 hr after the medium was changed to serum-free medium ExCell302 (JRH Biosciences, Lenexa, KS). After 24 hr of incubation, the media were collected and precipitated with trichloroacetic acid. Precipitated protein was collected by centrifugation at 15,000 × g for 20 min, followed by washing with acetone twice; then, the pellet was redissolved in sodium dodecyl sulfate (SDS) sample buffer A (62.5 mM Tris-HCl [pH 6.8], 10% Glycerol, 2% SDS, 5% 2-mercaptoethanol (2ME), 10 μg/mL phenylmethylsulphonyl fluoride [PMSF]) and boiled for 5 min. HEK293 cells were harvested 48 hr after transfection and SDS sample buffer B (62.5 mM Tris-HCl [pH 6.8], 4 M Urea, 10% Glycerol, 2% SDS, 5% 2ME, 10 μg/mL PMSF) was added directly to the cell pellet. The pellet was then sonicated and boiled for 5 min.

These protein samples of cell supernatants and lysates were separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to nitrocellulose membrane. The nitrocellulose membrane was blocked with Tris-buffered saline containing 5% (w/v) skimmed milk and 0.05% (v/v) Tween 20, incubated with anti-PA-PLA₁α monoclonal antibody [Sonoda et al., 2002], and then treated with antirat IgG antibody conjugated with horseradish peroxidase. Proteins bound to the antibodies were

visualized with an enhanced chemiluminescence kit (ECL, Amersham Biosciences, Piscataway, NJ) by LAS4000 Luminescent Image Analyzer (Fujifilm, Tokyo, Japan) [Sonoda et al., 2002].

PA-PLA₁α Enzyme Activity Assay

PA-PLA₁α produces 2-acyl lysophosphatidic acid (LPA) and free fatty acid (FFA) concurrently from phosphatidic acid (PA) [Sonoda et al., 2002]. In the present study, the hydrolysis activity was determined measuring oleic acids, which are concurrently produced from dioleoyl PA by PA-PLA₁α. We added the supernatant from HEK293 cells transfected with WT, p.Ser154Ala, p.Cys246Ser, or p.His248Asn PA-PLA₁α to the medium including 100 μM PA. After 12 hr incubation at 37°C, the amount of oleic acids was measured with NEFA C-Test Wako test kit (Wako Chemicals Co., Osaka, Japan).

P2Y5 Activation Ability Assay

We cotransfected alkaline-phosphatase-tagged transforming growth factor-α (AP-TGFα) (kindly provided by Dr. Higashiyama, Ehime University, Japan) [Tokumaru et al., 2000], recombinant P2Y5 and WT, p.Ser154Ala, p.Cys246Ser, or p.His248Asn PA-PLA₁α to HEK293 cells, and we quantified free AP-TGFα induced by a disintegrin and metalloprotease (ADAM) in the HEK293 cells to examine the P2Y5 activation ability of LPA produced by mutant PA-PLA₁α. Cells were cultured in 100 μL of serum-free medium Opti-MEM (Gibco BRL, Grand Island, NY) in individual wells of a 96-well plate. After 24 hr of incubation, 80 μL of the conditioned medium in each well was transferred and AP activities in both the conditioned media and the transfected cells were measured using *p*-nitrophenyl phosphate (*p*-NPP). In the case of phorbol ester, 12-O-tetradecanoylphorbol-13-acetate (TPA)-stimulation, the transfected cells were treated with 100 nM 1 h before medium transfer. The AP reaction was performed in *p*-NPP buffer (5 mM *p*-NPP, 20 mM Tris-HCl (pH 9.5), 20 mM NaCl, and 5 mM MgCl₂) at 37°C for 1 hr and the increases in the reaction product, *p*-nitrophenol, were quantified by monitoring absorbance at 405 nm with VersaMax microplate reader (Molecular Devices, Sunnyvale, CA). The amount of AP-TGFα released was expressed as a ratio of AP activity in the conditioned media to total AP activity in each well.

Results

Clinical Findings

All six affected individuals in the five unrelated Japanese families showed features typical of ARH (Fig. 1A, B, E, H, K, and N). The patients were less than 10 years of age at the time of the study. Affected individuals had tightly curled hair, which grew slowly and stopped growing after a few inches. Their eyebrows and eyelashes were a little sparse to absent. Nails, teeth, sweating, and hearing were normal in all the affected individuals. Heterozygous carriers had normal hair. The pedigrees of all the families were consistent with autosomal recessive inheritance (Fig. 1C, F, I, L, and O).

Mutation Detection

Direct sequencing analysis of exons and intron-exon boundaries of *LIPH* revealed that affected members of Families A, B, D, and E were compound heterozygous for the two missense mutations

c.736T>A (p.Cys246Ser) and c.742C>A (p.His248Asn) (Fig. 1C, F, I, L, O). The affected individual in Family C was homozygous for c.736T>A. All the parents whose DNA was available for mutation search were heterozygous carriers of one of the two mutations (Fig. 1C, F, I, L, and O). We confirmed these *LIPH* mutations by MASA analysis (Fig. 1D, G, J, M, and P). Both amino acid residues altered by the two missense mutations were highly conserved among diverse species (Fig. 2A). One of the mutations was found in 4/200 normal unrelated alleles (100 healthy Japanese individuals) by direct sequence analysis (minor allele frequency, c.736T>A, 0.015 (3/200); c. 742C>A, 0.005 (1/200); combined genotype 0.02 (4/200)), although there was no control individual who had compound heterozygous or homozygous mutations (data not shown). No other pathogenic mutation was found in the entire exon or intron/exon borders of the *DSG4*, *LIPH* or *LPAR6* gene.

Haplotype Analysis

The haplotype block structure containing the *LIPH* gene was constructed using genotype data from the HapMap database (Fig. 3B). The haplotype block was represented by five haplotypes with >1% frequency (Fig. 3C). The haplotype of the chromosome containing the *LIPH* c.736T>A mutation was found to have resulted from parent-to-child transmission in all five families (Table 1). The chromosome containing the *LIPH* c.736T>A mutation had haplotype I (ATCAACCGGA), which is seen in 37.8% of the Han Chinese and ethnic Japanese populations. Likewise, we determined the haplotype of the chromosome containing the *LIPH* c.742C>A mutation in four families (A, B, D, E). The chromosome containing the *LIPH* c.742C>A mutation had haplotype III (GCTCGTGAGG), which is seen in 28.9%. Thus, these missense mutations c.736T>A (p.Cys246Ser) and c.742C>A (p.His248Asn) in Japanese patients appear to represent founder effects in this island nation.

Expression of PA-PLA₁α in Mammalian Cells

Immunoblot analysis revealed that transfection of p.Cys246Ser and p.His248Asn mutant constructs into HEK293 cells resulted in the secretion of 55-kDa mutant PA-PLA₁α at a level similar to that of the WT and of the p.Ser154Ala mutant (Fig. 4A). In addition, the same amounts of mutant PA-PLA₁α proteins were also recovered from the cell lysate. These results indicated that there was no significant difference in protein yield between WT and mutant PA-PLA₁α.

Analysis of PA-PLA₁α Hydrolytic Activity

The hydrolysis activity was determined measuring FFA which are concurrently produced from PA by PA-PLA₁α. The quantities of FFA produced by the p.Cys246Ser and p.His248Asn mutant *LIPH* constructs were similar to those by the mock and p.Ser154Ala mutant constructs, suggesting that the p.Cys246Ser and p.His248Asn mutant PA-PLA₁α had no hydrolytic activity (Fig. 4B).

P2Y5 Activation Ability of PA-PLA₁α Mutants

In this study, we cotransfected AP-TGFα, recombinant P2Y5 and WT, p.Ser154Ala, p.Cys246Ser, or p.His248Asn PA-PLA₁α constructs to HEK293 cells. To examine the P2Y5 activation potency of mutant PA-PLA₁α, AP-TGFα release into conditioned media via ADAM, which was triggered by activation of P2Y5, was

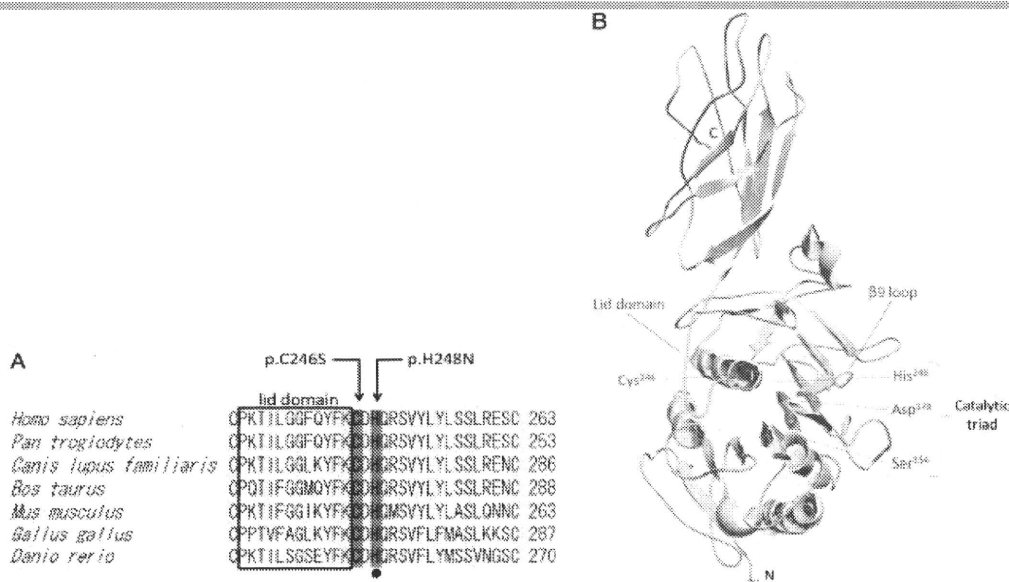


Figure 2. Conservation of the mutated residues and the three-dimensional protein structure around the mutation sites. **A:** Multiple amino acid sequence alignments of PA-PLA₁α of diverse species. Amino acid residues Cys²⁴⁶ and His²⁴⁸ altered by the present two mutations are highly conserved among PA-PLA₁α of diverse species. Amino acid residues that are conserved between the seven species are shown in yellow. The 12 residues that comprise the lid domain are surrounded by a black rectangle. One of the amino acids of the catalytic triad, His²⁴⁸, is marked with a black dot. Cys²⁴⁶ and His²⁴⁸ are in red and indicated by arrows. **B:** The three-dimensional-structure model of PA-PLA₁α protein. Cys²⁴⁶ and His²⁴⁸ residues are in red. Lid domain and β9 loop are in green. Catalytic triad consists of Ser¹⁵⁴ (purple), Asp¹⁷⁸ (purple) and His²⁴⁸. Cys²⁴⁶ forms intramolecular disulfide bonds on the lid domain.

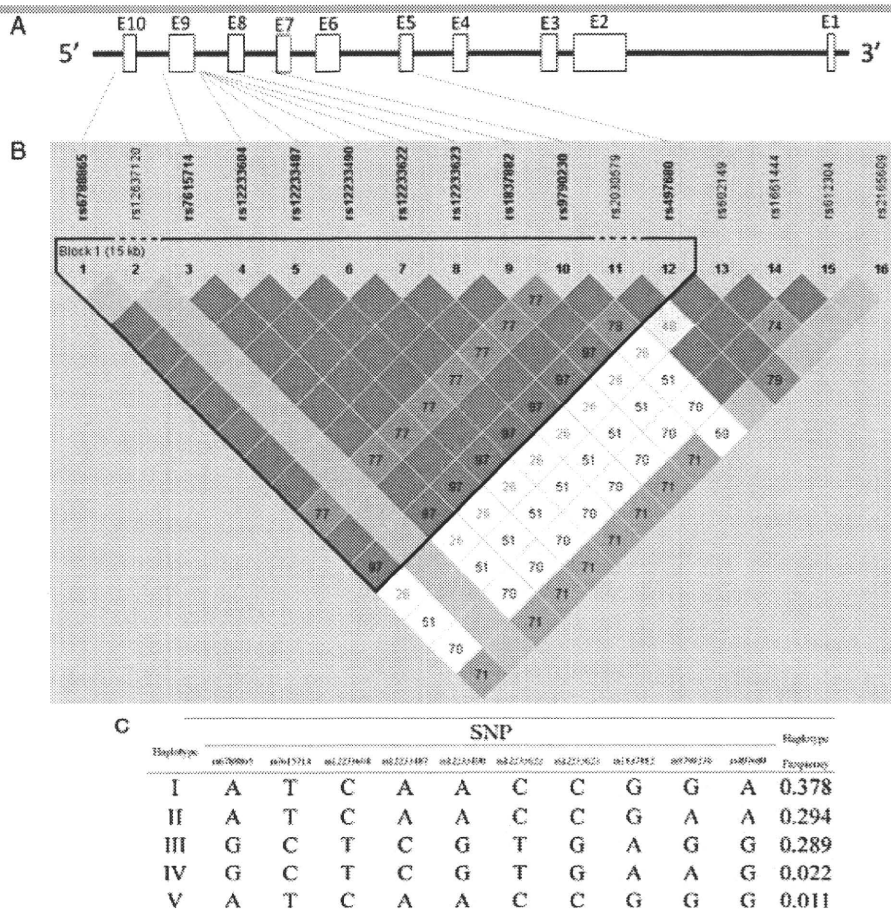


Figure 3. The linkage disequilibrium (LD) block and the haplotype structure around *LIPH* in Han Chinese and ethnic Japanese populations. *LIPH* structure (**A**) and the LD block within *LIPH* (**B**) were evaluated using genotype data from the HapMap database. **C:** The haplotype structure with 10 tag-SNPs was determined using Haploview.

Table 1. Identified Haplotype with the *LIPH* c.736T>A and c.742C>A Mutation

Family	Mutation	rs6788865	rs7615714	rs12233604	rs12233487	rs12233490	rs12233622	rs12233623	rs1837882	rs9790230	rs497680	Haplotype
A	c.736T>A	A/G	T/C	C/T	A/C	A/G	C/T	C/G	G/A	G/G	A/G	I/III
	c.742C>A	A/G	T/C	C/T	A/C	A/G	C/T	C/G	G/A	G/G	A/G	I/III
B	c.736T>A	A	T	C	A	A	C	C	G	G	A	I
	c.742C>A	G	C	T	C	G	T	G	A	G	G	III
C (homozygote)	c.736T>A	A	T	C	A	A	C	C	G	G	A	I
D	c.736T>A	A	T	C	A	A	C	C	G	G	A	I
	c.742C>A	G	C	T	C	G	T	G	A	G	G	III
E	c.736T>A	A/G	T/C	C/T	A/C	A/G	C/T	C/G	G/A	G/G	A/G	I/III
	c.742C>A	A/G	T/C	C/T	A/C	A/G	C/T	C/G	G/A	G/G	A/G	I/III

Nucleotide numbering starts at +1 corresponding to the A of the ATG initiation codon in the reference sequence AY093498.1 (www.hgvs.org/mutnomen). SNP, single-nucleotide polymorphism.

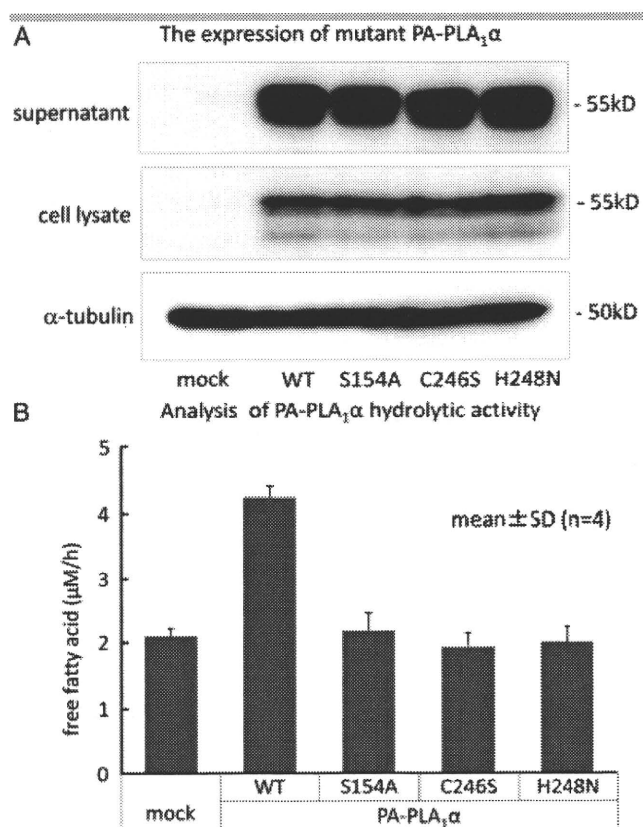


Figure 4. Expression of PA-PLA₁α in HEK293 cells and its hydrolytic activity. **A:** Expression of mutant PA-PLA₁α in HEK293 cells. HEK293 cells were transfected with wild-type (WT), p.Ser154Ala (S154A), p.Cys246Ser (C246S), and p.His248Asn (H248N) *LIPH* cDNA, and the expression level of PA-PLA₁α protein derived from the constructs in cell culture supernatant (upper panel) and cells (middle panel) were evaluated by Western blot. There were no significant differences in PA-PLA₁α protein expression levels among cells transfected with WT, S154A, C246S, and H248N. α-tubulin expression was used as a standard to assess the total amount of proteins from cell lysate loaded on the gel (lower panel). **B:** Because PA-PLA₁α hydrolyzes the free fatty acid (FFA) from PA, we monitored the levels of FFA to determine whether there is a difference in the PA-PLA₁α hydrolytic activity among WT and the three mutants of PA-PLA₁α. After 12-hr incubation of the supernatant from HEK293 cells expressing WT, S154A, C246S, or H248N PA-PLA₁α, with a medium including 100 μM PA, the levels of FFA hydrolyzed by C246S and H248N mutant PA-PLA₁α were significantly lower than that by WT PA-PLA₁α and similar to those produced by supernatant from HEK293 cells transfected with control S154A mutant and an empty vector (mock).

quantified using *p*-NPP as a substrate for AP. The free AP-TGFα from the P2Y5 mock transfected (P2Y5-) cells transfected with the WT form of PA-PLA₁α was more abundant than that from the P2Y5- cells transfected with empty vector, which indicated that the HEK293 cells had the ability to shed TGFα mediated by intrinsic LPA receptor at some level (Fig. 5A). AP-TGFα release from P2Y5 positive (P2Y5+) cells expressing the WT PA-PLA₁α was remarkably increased compared with mock or mutant PA-PLA₁α. There were no significant differences between the data obtained with cells expressing the mutants and the empty vector (Fig. 5A). All the cells expressing AP-TGFα responded equally to TPA, confirming that expression of P2Y5 and PA-PLA₁α did not affect PKC-dependent AP-TGFα release (Fig. 5B). These data clearly indicated that these mutations resulted in the loss of P2Y5 activation activity of PA-PLA₁α.

Discussion

The human *LIPH* gene encodes PA-PLA₁α, which is a member of the membrane-associated phosphatidic acid-preferring phospholipase A₁ [Hiramatsu et al., 2003; Jin et al., 2002; Sonoda et al., 2002]. Similar to other phospholipase A₁, PA-PLA₁α has N-terminal domains that are essential for catalytic activity. Three amino acid residues, Ser¹⁵⁴, Asp¹⁷⁸, and His²⁴⁸, which form the putative catalytic triad, are located in the N-terminal domains [Aoki et al., 2007; Jin et al., 2002; Kubiak et al., 2001; Sonoda et al., 2002] (Fig. 2B). PA-PLA₁α has a β9 loop (the 13 amino acids from p.206 to 218) and a short lid domain (the 12 amino acids from p.234 to 245), each of which is considered a crucial structure for substrate recognition [Aoki et al., 2007; Carriere et al., 1998; Sonoda et al., 2002]. In addition, well-conserved cysteine residues including Cys²⁴⁶, which form intramolecular disulfide bonds, are in the N-terminal domains.

We performed *DSG4*, *LIPH*, and *LPAR6* gene mutation analysis and identified two prevalent missense mutations in the *LIPH* gene in the five independent Japanese ARH families. One mutation c.736T>A leads to an amino acid change within conserved cysteine residue that forms intramolecular disulfide bonds on the lid domain (p.Cys246Ser) (Fig. 2). The other mutation c.742C>A results in alteration of one amino acid of the catalytic triad (p.His248Asn) (Fig. 2B). These two residues, Cys²⁴⁶ and His²⁴⁸, are highly conserved among *LIPH* of diverse species (Fig. 2A), suggesting that they play a critical role in enzyme activity. We speculate that these mutations drastically affect PA-PLA₁α activity.

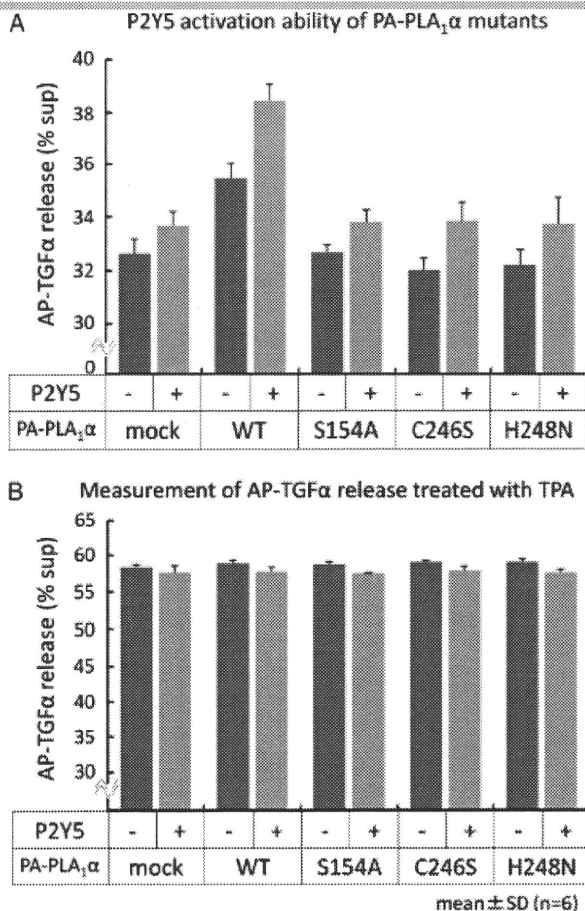


Figure 5. P2Y5 activation ability of PA-PLA₁α mutants. To monitor P2Y5 activation level by mutant and wild-type (WT) PA-PLA₁α, we used *p*-nitrophenyl phosphate as a substrate for cleavage of AP-TGFα and measured the amount of AP-TGFα released from the HEK293 cells. **A:** The amount of free AP-TGFα produced by P2Y5 mock-transfected (P2Y5⁻) cells that were also transfected with WT PA-PLA₁α is significantly greater than that produced by P2Y5⁻ cells transfected with an empty vector (mock). This indicates that HEK293 cells act to shed AP-TGFα, an activity that might be mediated by intrinsic LPA receptors. The amounts of AP-TGFα released from P2Y5-transfected (P2Y5⁺) cells expressing p.Ser154Ala (S154A), p.Cys246Ser (C246S), or p.His248Asn (H248N) mutant PA-PLA₁α and P2Y5⁺ cells transfected with an empty vector (mock) are significantly lower than that from P2Y5⁺ cells expressing WT PA-PLA₁α. **B:** TPA sheds AP-TGFα independently from the P2Y5 pathway. Effects of the TPA-induced shedding of AP-TGFα are similar in all the cells.

So far, 14 *LIPH* gene mutations have been reported, four of which are prevalent [Ali et al., 2007; Horev et al., 2009; Jelani et al., 2008; Kamran-ul-Hassan Naqvi et al., 2009; Kazantseva et al., 2006; Nahum et al., 2009; Naz et al., 2009; Pasternack et al., 2009; Petukhova et al., 2009; Shimomura et al., 2009a,b,c]. One prevalent mutation, 985-bp deletion including exon 4 and the flanking introns, was detected in a large number of ARH patients from two ethnic groups, the Chuvash and Mari, in the Volga-Ural region of Russia [Kazantseva et al., 2006]. The ancestors of the Chuvash population settled in territory occupied by ancestral Mari populations. To determine the frequency of the mutant allele, they tested 2,292 chromosomes in the populations and found the *LIPH* deletion in populations of Chuvash (mutant allele frequency $P = 0.033$) and Mari (mutant allele frequency $P = 0.030$) origin. The mutant allele was restricted to these

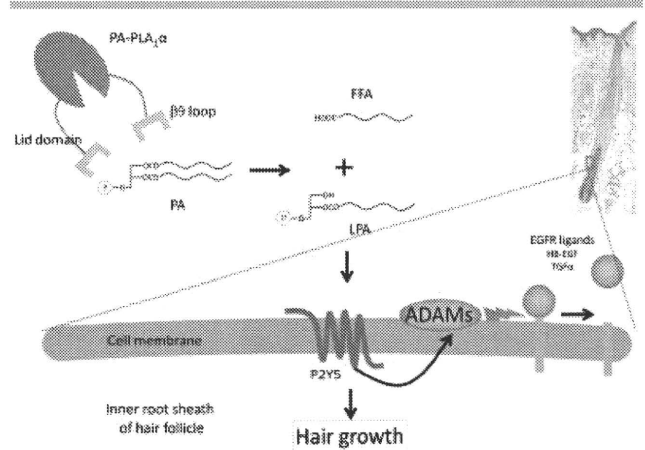


Figure 6. Schematic signaling pathways of LPA produced by PA-PLA₁α via the P2Y5 receptor. PA-PLA₁α hydrolyzes PA and produces LPA and FFA. LPA works as a ligand for P2Y5, a membrane-bound G-protein-coupled receptor. It has been documented that ADAM activation by P2Y5 results in ectodomain shedding of cell surface proteins including those of the EGF ligand family, such as HB-EGF and TGFα. These signal pathways are speculated to regulate proliferation and differentiation of inner root sheath cells of hair follicles. Abbreviations: PA, phosphatidic acid; FFA, free fatty acid; LPA, 2-acyl lysophosphatidic acid; ADAM, a disintegrin and metalloprotease; EGF, epidermal growth factor; HB-EGF, heparin binding EGF-like growth factor; TGFα, transforming growth factor-α.

two populations and was not found in other Finno-Ugric populations or Russian populations from distant geographic regions [Kazantseva et al., 2006].

A deletion mutation exon7_8del has been identified in five consanguineous Pakistani families and 1 Guyanese family [Jelani et al., 2008; Petukhova et al., 2009; Shimomura et al., 2009b, 2009c]. A small deletion mutation 659_660delTA has been identified in several consanguineous Pakistani families and 1 Guyanese family [Jelani et al., 2008; Petukhova et al., 2009; Shimomura et al., 2009b,c]. Both mutations were defined as founder mutations shared in families from Pakistan and Guyana by haplotype analysis using microsatellite markers close to the *LIPH* gene [Jelani et al., 2008; Petukhova et al., 2009; Shimomura et al., 2009b,c]. In fact, these Guyanese families with ARH were descended from people who had come from India about 100 years ago, and it is plausible that both mutations originated from the Indian population [Shimomura et al., 2009c]. However, neither exon7_8del nor 659_660delTA mutations were detected in healthy control individuals of Pakistani origin and their minor allele frequencies were thought to be low in the Pakistani population [Jelani et al., 2008; Shimomura et al., 2009b].

All six of the Japanese ARH patients from the five families in the present study were compound heterozygous for c.736T>A (p.Cys246Ser) and c.742C>A (p.His248Asn) or homozygous for c.736T>A (p.Cys246Ser). c.736T>A (p.Cys246Ser) was found in all five families, and c.742C>A (p.His248Asn) was detected in four of the five families. Most recently, these missense mutations were identified in three Japanese ARH families [Shimomura et al., 2009a]. One family carries two heterozygous missense mutations, c.736T>A and c.742C>A, and the other two families are homozygous for the mutation c.736T>A. Thus, the missense mutations c.736T>A (p.Cys246Ser) and c.742C>A (p.His248Asn) are both suggested to be highly prevalent *LIPH* mutations in the Japanese population. In the previous article, however, screening assays with restriction enzymes excluded the existence of both

mutations in 100 unrelated healthy control individuals (200 alleles) of Japanese origin [Shimomura et al., 2009a]. In this study, in contrast, we used direct sequences and MASA analysis and identified these mutations in four alleles out of 200 unrelated control alleles (100 individuals) (minor allele frequency of c.736T>A, 3/200 $P=0.015$; c. 742C>A, 1/200 $P=0.005$; combined genotype, 4/200 $P=0.020$). In addition, the present haplotype analysis revealed that the mutant alleles with c.736T>A and those with c.742C>A had specific haplotypes, respectively, which suggests that they derive from their own independent founders (Fig. 3, Table 1). From these results, we consider that the *LIPH* mutations c.736T>A (p.Cys246Ser) and c.742C>A (p.His248Asn) are extremely prevalent founder mutations for ARH in the Japanese population.

Previously, several deletion mutations and four missense mutations were reported in the *LIPH* gene [Ali et al., 2007; Horev et al., 2009; Jelani et al., 2008; Kamran-ul-Hassan Naqvi et al., 2009; Kazantseva et al., 2006; Nahum et al., 2009; Naz et al., 2009; Pasternack et al., 2009; Petukhova et al., 2009; Shimomura et al., 2009a,b,c]. In previous cases, ARH patients exhibited wide variability in the hypotrichosis phenotype, although most patients showed wooly hair during early childhood [Shimomura et al., 2009b]. Even ARH patients with identical *LIPH* gene mutations showed a wide variation in phenotype [Shimomura et al., 2009b]. In our cases, all the affected individuals had sparse, curled hair that grew slowly from birth and then stopped growing after reaching a few inches. There are no significant differences in clinical features between families and patients. We cannot exclude the possibility that differences in phenotype will emerge in the future, because our patients were still less than 10 years of age. The clinical features of the five families presented here are similar to those of families with the other mutations in the *LIPH* gene, and no apparent genotype/phenotype correlation was observed between the patients with deletion mutations and those with missense mutations.

PA-PLA₁α hydrolyzes PA and produces LPA and FFA concurrently [Sonoda et al., 2002]. The LPA that is produced by PA-PLA₁α acts as a ligand for P2Y₅, one of the G-protein-coupled receptors (GPCRs), which has been identified as another causative gene for human hair growth deficiency [Pasternack et al., 2008; Shimomura et al., 2008]. It has been documented that ADAM activation by GPCRs introduces the ectodomain shedding of cell surface proteins, including the epidermal growth factor (EGF) ligand family whose members include heparin-binding EGF-like growth factor (HB-EGF) and TGFα [Ohtsu et al., 2006] (Fig. 6).

In this study, we performed two different in vitro PA-PLA₁α enzyme activity analyses. One involved analyzing PA-PLA₁α hydrolytic activity by measuring FFA (unpublished data). The p.Cys246Ser and p.His248Asn mutants showed complete abolition of PA-PLA₁α hydrolytic activity, comparable with supernatant of cells transfected with the empty vector only or with the control loss-of-function mutant carrying p.Ser154Ala. The other involved analyzing the P2Y₅ activation ability of LPA produced by PA-PLA₁α by assaying free AP-TGFα (unpublished data). In this analysis, the p.Cys246Ser and p.His248Asn mutant PA-PLA₁α had no ability to activate P2Y₅. These results clearly indicated that a loss of PA-PLA₁α function leads to defective activation of P2Y₅ by LPA, resulting in ARH phenotype in ARH patients with *LIPH* mutations. Thus, complete loss of P2Y₅ activation due to reduced LPA is thought to be involved in the pathogenesis of ARH.

While we were preparing the manuscript, Pasternack et al. [2009] reported that PA-PLA₁α derived from mutants with

c.403_409 duplication frameshift mutation and in-frame mutations including c.280_369dup and c.527_628del did not show the enzymatic activity of converting PA to LPA in vitro, and that they did not activate P2Y₅. The results presented in this study completely agree with their results, although the assay system for enzymatic evaluation and P2Y₅ activation used by Pasternack et al. [2009] is quite different from ours. In addition, the affected amino acids in the mutant PA-PLA₁α analyzed in this study were quite different. Interestingly, our in vitro enzyme activity analysis revealed that the present two missense mutations strikingly affected the PA-PLA₁α activity as much as frameshift mutations and large deletion mutations like c.403_409 dup, c.280_369dup, and c.527_628del. These results were consistent with the fact that there is no significant difference in severity of hair loss between the present patients with missense mutations and affected individuals with frameshift mutations or large deletion mutations, c.403_409 dup, c.280_369dup, and c.527_628del. These results clearly indicated that the loss of PA-PLA₁α function caused by the two present mutations leads to defective activation of P2Y₅ by LPA and suggest that loss of P2Y₅ activation due to reduced LPA is involved in the pathogenesis of ARH.

Acknowledgments

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References

- Ali G, Chishti MS, Raza SI, John P, Ahmad W. 2007. A mutation in the lipase H (*LIPH*) gene underlie autosomal recessive hypotrichosis. *Hum Genet* 121: 319–325.
- Aoki J, Inoue A, Makide K, Saiki N, Arai H. 2007. Structure and function of extracellular phospholipase A1 belonging to the pancreatic lipase gene family. *Biochimie* 89:197–204.
- Barrett JC, Fry B, Maller J, Daly MJ. 2005. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 21:263–265.
- Carriere F, Withers-Martinez C, van Tilbeurgh H, Roussel A, Cambillau C, Verger R. 1998. Structural basis for the substrate selectivity of pancreatic lipases and some related proteins. *Biochim Biophys Acta* 1376:417–432.
- Hasegawa Y, Takeda S, Ichii S, Koizumi K, Maruyama M, Fujii A, Ohta H, Nakajima T, Okuda M, Baba S, Nakamura Y. 1995. Detection of K-ras mutations in DNAs isolated from feces of patients with colorectal tumors by mutant-allele-specific amplification (MASA). *Oncogene* 10:1441–1445.
- Hiramatsu T, Sonoda H, Takanezawa Y, Morikawa R, Ishida M, Kasahara K, Sanai Y, Taguchi R, Aoki J, Arai H. 2003. Biochemical and molecular characterization of two phosphatidic acid-selective phospholipase A1s, mPA-PLA1alpha and mPA-PLA1beta. *J Biol Chem* 278:49438–49447.
- Horev L, Tosti A, Rosen I, Hershko K, Vincenzi C, Nanova K, Mali A, Potikha T, Zlotogorski A. 2009. Mutations in lipase H cause autosomal recessive hypotrichosis simplex with wooly hair. *J Am Acad Dermatol* 61:813–818.
- Jelani M, Wasif N, Ali G, Chishti M, Ahmad W. 2008. A novel deletion mutation in *LIPH* gene causes autosomal recessive hypotrichosis (LAH2). *Clin Genet* 74:184–188.
- Jin W, Broedl UC, Monajemi H, Glick JM, Rader DJ. 2002. Lipase H, a new member of the triglyceride lipase family synthesized by the intestine. *Genomics* 80:268–273.
- Kamran-ul-Hassan Naqvi S, Raza SI, Naveed AK, John P, Ahmad W. 2009. A novel deletion mutation in the phospholipase H (*LIPH*) gene in a consanguineous Pakistani family with autosomal recessive hypotrichosis (LAH2). *Br J Dermatol* 160:194–196.
- Kazantseva A, Goltsov A, Zinchenko R, Grigorenko AP, Abrukova AV, Moliaka YK, Kirillov AG, Guo Z, Lyle S, Ginter EK, Rogaev EI. 2006. Human hair growth