human skin, or young human skin treated *ex vivo* with hMMP-1/V94G [5].

To complement AFM data and electron microscopy imaging, we performed quantitative biochemical analysis of collagen fibril fragmentation. Fragmentation was quantified by measurement of susceptibility of insoluble collagen fibrils to chymotrypsin-catalyzed proteolysis [8]. Fragmented collagen levels were two-fold higher in K5-hMMP-1/V94GTg mice, compared to non-Tg littermates (Fig. 2E).

We further investigated fibroblast-collagen fibril interactions by examining immunostaining of β1 integrin, which is a component of all collagen-binding integrins. Fibroblasts in non-TG mouse skin, displayed a spread morphology and were strongly positive for β1 integrin immunostaining. Bright punctate staining, representing focal adhesions, was observed on the periphery of the cells. In contrast, fibroblasts in K5-hMMP-1/V94GTg mouse skin displayed a collapsed morphology, and significantly less β1 integrin staining, consistent with reduced interactions with collagen fibrils (Fig. 2F).

MMP-1 is elevated in aged human skin [1,4]. The epidermis is the primary source of MMP-1 induced by acute UV irradiation [4]. MMP-1 protein is secreted and transits to the dermis where it degrades collagen fibrils in the dermis [4,9]. K5-hMMP-1/V94GTg mice expressing active hMMP-1 in the epidermis recapitulate some of the prominent features of aged human skin, including fragmentation/disorganization of collagen fibrils, reduced fibroblast attachment, and contracted fibroblast morphology. These findings support the concept that elevated MMP-1 is a critical mediator of age-related decline of collagen fibril structure. K5-hMMP-1/V94GTg mice may be a useful model to help identify safe and effective MMP-1 inhibitors to reduce the detrimental effects of human dermal aging.

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Letter to the Editor

Mutation study for 9 genes in 23 unrelated patients with autosomal recessive congenital ichthyosis in Japan and Malaysia

Keywords:
Autosomal recessive congenital ichthyosis; Malaysia; Mutation analysis

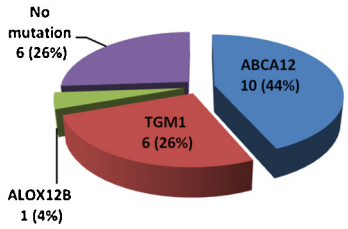
Autosomal recessive congenital ichthyosis (ARCI) is heterogeneous group of ichthyosis and consists of Harlequin ichthyosis (HI, MIM #242500), lamellar ichthyosis (LI, MIM #242304) and congenital ichthyosiform erythroderma (CIE, MIM #242100). Harlequin ichthyosis shows the severest phenotype and used to be almost fatal at birth [1]. Even milder types of ARCI severely influence quality of life of patients. To date, mutations in 9 causative genes have been reported in ARCI, including ATP-binding cassette, subfamily a, member 12 (*ABCA12*) [2,3], transglutaminase 1 (*TGM1*) [2,3], arachidonate lipoxygenase 3 [2,3], arachidonate 12-lipoxygenase, 12R type (*ALOX12B*) [2,3], NIPA-like domain containing 4 [2,3], cytochrome P450, family 4, subfamily F, polypeptide 22 [2,3], lipase, family member N [2,3], patatin-like phospholipase domain-containing 1 [3] and ceramide synthase 3 [4]. However, mutations in these genes have been found in about 78% of ARCI patients [2].

In this study, we performed genetic analyses in 23 unrelated patients with ARCI. Sixteen out of 23 patients were from Japan, and 7 patients from Malaysia. Clinically and histopathologically, 3 patients were diagnosed as HI, 2 patients as LI, and 18 patients as CIE.

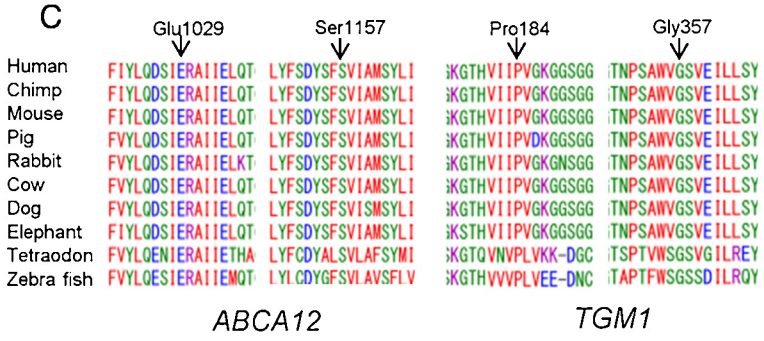
All experiments in this study were approved by Medical Ethical Committee of Kurume University School of Medicine, and conducted according to Declaration of Helsinki Principles. Written informed consent was obtained from each individual.

Mutation and polymorphism analyses of 9 causative genes were performed, basically according to the methods described previously [5]. Briefly, genomic DNA was extracted from peripheral blood and PCR-direct sequencing was performed for all exons and their flanking intron boundaries in all 9 genes. Primer sequences for all 9 genes are shown in Supplemental data (Table S1). Primers which we designed using online Primer3 plus tool are indicated by red-colored letters (Table S1) [6]. We detected mutations in 17

A



C



B

No	Pheno-types	origins	Genes	Mutations
1	HI	Malaysia (Chinese)	ABCA12	c.2285T>A (p.Leu762X) / c.6858delA*1
2	HI	Malaysia (Chinese)	ABCA12	c.859C>T (p.Arg287X)*2/ **c.3085G>A (p.Glu1029Lys)**
3	HI	Malaysia (Malay)	ABCA12	c.3295-1G>A / -
4	LI	Japan	TGM1	c.374delA*3/ (homozygous)
5	LI	Malaysia (Malay/Chinese)	TGM1	c.379C>T (p.Arg127X)*4/ c.2290delC
6	CIE	Japan	TGM1	c.160C>T(p.Arg54X)*5/ c.1187G>T(p.Arg396Leu)*6 : rs121918721
7	CIE	Japan	TGM1	c.700C>T (p.Gln234X) / c.866A>C (p.Asn289Thr)*7: rs12198730
8	CIE	Malaysia (Malay)	TGM1	c.420A>G (p.Ile140Met)*8: rs139208806 / **c.1070G>A (p.Gly357Asp)**
9	CIE	Japan	ABCA12	c.4723A>C (p.Thr1575Pro)*9/ c.6031delG*9
10	CIE	Japan	ABCA12	c.2956C>T (p.Arg986Trp)*9: rs199499787 / c.5940-1G>C*9
11	CIE	Japan	ABCA12	c.4139A>G (p.Asn1380Ser)*10: rs28940269 / c.5128+3A>G*9
12	CIE	Japan	ABCA12	c.4723A>C(p.Thr1575Pro)*9 / c.4951G>A (p.Gly1651Ser)*10: rs28940568
13	CIE	Japan	ABCA12	c.2956C>T (p.Arg986Trp)*9 (homozygous)
14	CIE	Japan	ABCA12	**c.3470C>T (p.Ser1157Ile)** / -
15	CIE	Japan	ABCA12	c.3926delA / -
16	CIE	Malaysia (Malay)	ALOX12B	c.552delA / c.940delT
17	CIE	Malaysia (Indian)	TGM1	**c.550C>T (p.Pro184Ser)** : rs200517023 / c.984+1G>A*5

*1 Scott et al. (2013), *2 Castiglia et al. (2009), *3 Akiyma et al. (2003), *4 Huber et al. (1997), *5 Herman et al. (2009), *6 Laiho et al. (1997), *7 Yang et al. (2001), *8 Zang et al. (2012), *9 Fukuda et al. (2012), *10 Lefevre et al. (2003).

Fig. 1. The results of mutation analyses of 23 patents with ARCI and sequence alignment of missense mutations in 9 species. (A) Histogram presentation of the results of mutation analyses. (B) Table showing all data for patients and mutations. Asterisks (*) indicate reported mutations, underlined mutations had been reported by our group (Fukuda et al. [5]) in ARCI, and red letters are missense mutations. (C) Sequence alignment of missense mutations in *ABCA12* and *TGM1*. All 4 new missense mutations are highly conserved in 9 species. Arrows indicate the positions of residues of missense mutations.

(74%) of 23 patients. Mutations on both alleles were detected in 14 of 17 patients, while only one mutation could be detected in 3 patients. Ten (44%) of 17 patients showed mutations in *ABCA12*, 6 (26%) patients in *TGM1*, and one (4%) patient in *ALOX12B* (Fig. 1A). Eleven unreported mutations in ARCI were identified in this study and 4 out of 11 were missense mutations, 2 were in *ABCA12* and 2 in *TGM1*, (Fig. 1B). None of these 4 missense mutations were found in 100 control chromosomes selected to match the same ethnic background. Sequence alignment showed that the 4 missense mutations were conserved in nine different species (Fig. 1C). We identified 11 missense mutations in the present study and checked all by single-nucleotide polymorphisms (SNPs) database in National Center for Biotechnology Information (NCBI) web page and 7 of which have been reported as SNPs in the SNP database of the NCBI (Fig. 1B).

Japanese CIE patients most frequently showed *ABCA12* mutations, while Malaysian CIE patients more frequently showed *TGM1* mutations. These results were consistent with those in previous studies that most Japanese CIE patients showed *ABCA12* mutations [7], although no study of ARCI has been performed for Malaysian