CXCL8 in the NOD1 shRNA-expressing cells is mediated by such receptors.

In summary, to our knowledge this is the first report demonstrating that the intracellular receptor NOD1 is functional expressed in human keratinocytes, suggesting that NOD1 may be involved in cutaneous innate immunity. Further studies are needed to understand the contribution of intracellular innate immune receptors to cutaneous host defense.

CONFLICT OF INTEREST

The authors state no conflict of interest.

ACKNOWLEDGMENTS

We thank Joel Whitfield for help with the ELISAs and Peter Kuffa for lab assistance. This study was supported by NIH grant R01 DK61707 and a Heisenberg fellowship of the Deutsche Forschungsgemeinschaft (DFG) given to J Harder.

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Prevalent and Rare Mutations in the Gene Encoding Filaggrin in Japanese Patients with Ichthyosis Vulgaris and Atopic Dermatitis

Journal of Investigative Dermatology (2009) **129,** 1302–1305; doi:10.1038/jid.2008.372; published online 27 November 2008

TO THE EDITOR

Mutations in the gene encoding filaggrin (*FLG*) were identified as the underlying cause of ichthyosis vulgaris (IV; OMIM #146700) and also shown to predispose to atopic dermatitis (AD; Palmer *et al.*, 2006; Smith *et al.*, 2006). Although *FLG* is considerably difficult to analyze because of its large size (>12 kb) and highly repetitive nature, PCR strategy that permits routine and comprehensive sequencing of the entire *FLG* has been developed recently (Sandilands *et al.*, 2007).

Using this methodology, we have identified four prevalent *FLG* mutations in Japanese patients with IV (Nomura *et al.*, 2008). We also demonstrated that *FLG* mutations were significantly associated with AD and the frequency of these *FLG* mutations observed in our Japanese AD cohort was about 20%. However, the frequency in our cohort

Abbreviations: AD, atopic dermatitis; FLG, filaggrin; IV, ichthyosis vulgaris

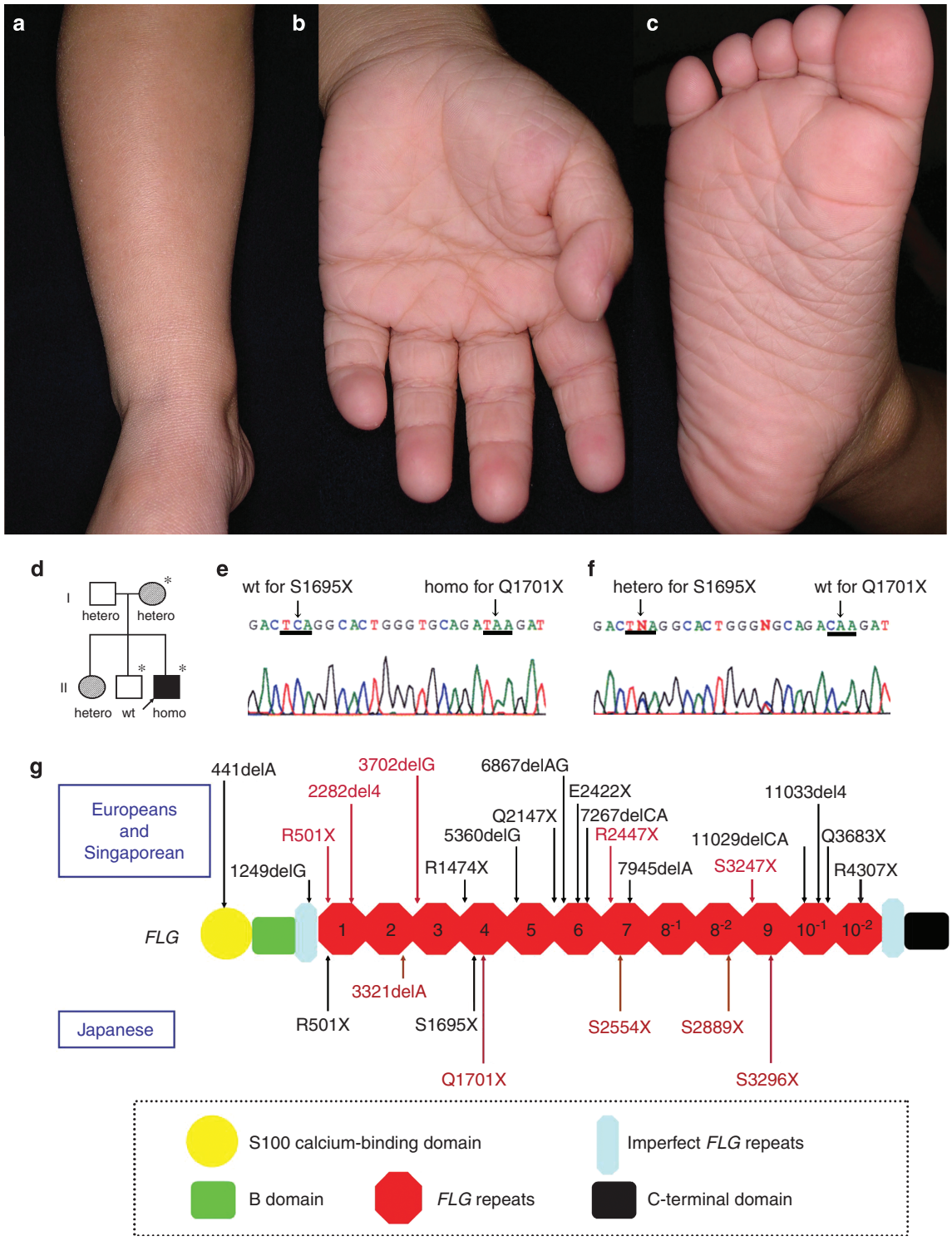

Figure 1. Clinical features and results of mutation analysis. (**a**) Fine scaling clearly visible on the proband's leg. (**b**, **c**) He also showed marked palmoplantar hyperlinearity. (**d**) A family tree of the ichthyosis vulgaris family shows the semidominant inheritance pattern. Solid symbols refer to the marked ichthyosis vulgaris presentation; cross-hatched symbols refer to the milder ichthyosis vulgaris presentation. In addition, the proband, his mother, and sister had concomitant dermatologist-diagnosed atopic dermatitis (*). wt, wild type for Q1701X; hetero, heterozygous; homo, homozygous. (**e**, **f**) A homozygous transition mutation c.5101C>T was identified in the proband, resulting in Q1701X. A heterozygous transition mutation c.5084C>G was identified in one Japanese individual in the control population, resulting in S1695X. Mutation S1695X is located only six amino acids upstream from Q1701X. (**g**) Loss-of-function *FLG* mutations are shown in a schematic of profilaggrin. Mutations shown in red are prevalent; those in black are rare. Some individuals have duplication of the 8th and/or 10th filaggrin repeat(s). Duplicated filaggrin repeats are represented as 8-1, 8-2, 10-1, and 10-2.

was still lower than that seen in analogous European case series, where it is up to 48% (Barker *et al.*, 2007; Sandilands *et al.*, 2007). Furthermore, it was reported that up to 37% of Japanese patients with AD had concomitant IV (Uehara and Hayashi, 1981; Uehara and Miyauchi, 1984). Taken together, there might be further prevalent *FLG* mutations to be discovered in the Japanese population. Here we have studied a further Japanese family with IV and identified two further *FLG* mutations.

A newly recruited Japanese family with IV was studied. The proband, a one-year-old Japanese boy, showed marked scaly dry skin on the extensor limbs and trunk (Figure 1a). Marked palmoplantar hyperlinearity was also evident (Figure 1b and c). A diagnosis of IV was made from these clinical observations. His mother and sister also showed scaly dry skin and palmoplantar hyperlinearity, but the clinical severity was mild compared to the proband (Figure 1d). Therefore, the inheritance pattern seemed semidominant. The proband, his mother, and his brother had concomitant AD.

The medical ethical committee at Hokkaido University Graduate School of Medicine approved all the studies. The study was conducted according to the Declaration of Helsinki Principles. Participants or their legal guardians gave their written informed consent. Following informed consent, genomic DNA from all family members was extracted from peripheral blood according to standard procedures. Initially, all family members were screened for five *FLG* mutations identified in Japanese population so far, R501X, 3321delA, S2554X, S2889X and S3296X, by restriction enzyme digestion, fluorescent PCR, and direct DNA sequencing as described previously (Nomura *et al.*, 2007, 2008; Hamada *et al.*, 2008). However, all individuals were wild type for these variants. Thus, we carried out full sequencing of the *FLG* as described previously (Sandilands *et al.*, 2007), which led to the identification of a previously unreported nonsense mutation Q1701X in repeat 4 in the present family (Figure 1e). The proband turned out to be homozygous for this truncation mutation and his non-consanguineous parents and his sister heterozygous, whereas his brother wild type (Figure 1d). It was also confirmed that they carry no pathogenic mutations in the other *FLG* repeats. Then, we screened 118 unrelated Japanese patients with AD and 134 unrelated Japanese control individuals for Q1701X by direct DNA sequencing. The diagnosis of AD in our case series was made by experienced dermatologists, according to the AD diagnostic criteria by Hannifin and Rajka (1980). Notably, mutation Q1701X was also identified in two Japanese patients with AD (1.7%), which brings the total number of recurrent *FLG* mutations so far identified in Japanese population to five.

During the screening for Q1701X, we identified another previously unreported *FLG* mutation, S1695X, which is located only six amino acids upstream from Q1701X, in the general Japanese control population (Figure 1f). We screened 33 Japanese patients with IV and 118 with AD for S1695X, but all patients were wild type for this mutation. Only one heterozygote was identified in the control population. Therefore, S1695X seems to be an extremely rare *FLG* mutation in Japanese individuals. The control individuals had not been examined in relation to AD or IV status, that is, they were population controls rather than ''hypernormal'' controls, so no clinical details about the individual carrying S1695X are available. In total, there are at least seven *FLG* variants in the Japanese population, including five that are prevalent and two that are quite rare.

The *FLG* genotype data in the Japanese AD case series and ethnically matched population control series are summarized in Table 1. In this study, case–control association analyses were performed by using Pearson's r^2 statistics, as previously described (Palmer *et al.*, 2006). All alleles were observed to be in normal Hardy–Weinberg equilibrium. Here we demonstrate that about 25% of patients in our Japanese AD case series carry one or more of these seven *FLG* mutations (combined minor allele frequency = 0.127, $n = 236$) and these variants are also carried by 4% of general Japanese control individuals (combined minor allele frequency = 0.019, $n = 268$). There is significant statistical association between the seven *FLG* mutations and AD (r^2 $P = 1.75 \times 10^{-6}$). Moreover, AD was manifested in heterozygous carriers of these *FLG* mutations with a Fisher's exact test odds ratio for AD of 6.8 (95% CI 2.5–18.5, $P = 3.7 \times 10^{-5}$), implying a causal relationship between *FLG* mutations and AD. Taken together, these data strongly suggest that skin barrier impairment because of reduced filaggrin expression is important in the pathogenesis in AD.

To date, 24 *FLG* mutations, including the two identified in this study, have been reported in the European, Japanese, and Singaporean populations (Sandilands *et al.*, 2007; Chen *et al.*, 2008; Nomura *et al.*, 2008). Interestingly,

Table 1. Atopic dermatitis case–control association analysis for *FLG* null variants in Japan

	R501X		3321delA		S1695X		Q1701X		S2554X		S2889X		S3296X		Combined	
Genotypes	Controls	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls	Cases
AA	134	118	133	113	133	118	134	116	133	112	132	105	134	114	129	91
Aa	0	0	1	5	1	0	0	2	1	6	2	13	0	4	5	24
aa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
Total	134	118	134	118	134	118	134	118	134	118	134	118	134	118	134	118

For combined genotype, r^2 $P = 1.75 \times 10^{-6}$; Fisher's exact test odds ratio=6.8 (95% CI 2.5–18.5).

mutations found in Japanese are different from those found in Europeans and Singaporean (Figure 1g), except in one case of the common European R501X mutation occurring as a very rare mutation on a different haplotype in the Japanese population (Hamada *et al.*, 2008). These observations imply that every population is highly likely to have a unique set of *FLG* mutations.

In conclusion, we have identified two further *FLG* mutations in the Japanese population. We also showed that at least about 25% of Japanese patients with AD carried one or more of *FLG* mutations. As we have sequenced more than 30 Japanese patients with IV, there is now little possibility that further highly prevalent mutations underlie the Japanese population. Taking the high frequency (up to 37%) of concomitant IV in patients with AD into account, however, it is still possible that there might be further multiple low-frequency *FLG* mutations to be discovered in the Japanese population. Further *FLG* mutation analysis will be necessary to understand the more precise genetic architecture of filaggrin-related AD in Japan.

CONFLICT OF INTEREST

Irwin McLean has filed patents relating to genetic testing and therapy development aimed at the filaggrin gene.

ACKNOWLEDGMENTS

We thank the patients and their families for their participation. We also thank Ai Hayakawa for the fine technical assistance. This work was supported in part by Grants-in-Aid from the Ministry of Education, Science, Sports, and Culture of Japan to M Akiyama (Kiban B 20390304). Filaggrin research in the McLean laboratory is supported by grants from The British Skin Foundation; The National Eczema Society; The Medical Research Council (reference number G0700314); A*STAR, Singapore; and donations from anonymous families affected by eczema in the Tayside region of Scotland.

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"White" Nevi and "Red" Melanomas: Association with the RHC Phenotype of the *MC1R* Gene

Journal of Investigative Dermatology (2009) **129,** 1305–1307; doi:10.1038/jid.2008.378; published online 4 December 2008

TO THE EDITOR

In 2002, we reported on three patients presenting with melanocytic nevi lacking pigmentation, which we named "white" dysplastic melanocytic nevi (DMN) due to their peculiar clinical appearance of white to pale red macules with accentuated skin markings and a silvery "shining" when observed with tangential light (Zalaudek *et al.*, 2002). Notably, all three patients had melanoma, and in one patient white DMN were associated with two primary amelanotic melanomas (AMMs).

We present herein a 25-year-old woman (skin type I, red hair, and blue eyes), who sought consultation for a mole check. Clinical examination revealed, besides approximately 30 slightly atypical light brown nevi on

Abbreviations: AMM, amelanotic melanoma; DMN, dysplastic melanocytic nevi; RHC, red hair color