

Japanese-Specific Filaggrin Gene Mutations in Japanese Patients Suffering from Atopic Eczema and Asthma

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TO THE EDITOR

Mutations in *FLG*, the gene encoding profilaggrin/filaggrin, are the underlying cause of ichthyosis vulgaris (OMIM 146700) and an important predisposing factor for atopic eczema (AE) (Sandilands *et al.*, 2007). *FLG* mutations are also significantly associated with asthma with AE mainly in the European population (Rodríguez *et al.*, 2009; van den Oord and Sheikh, 2010). The presence of population-specific *FLG* mutations has been reported in both the European and Asian races (Nomura *et al.*, 2007; Sandilands *et al.*, 2007). To clarify whether *FLG* mutations are a predisposing factor for asthma in the non-European population, we initially studied 172 Japanese AE patients (mean age, 24.8 ± 9.1 years) and 134 unrelated Japanese control individuals (healthy volunteers; mean age, 27.9 ± 6.0 years). All AE patients had been diagnosed based on widely recognized diagnostic criteria (Hanifin and Rajka, 1980). The majority of AE patients and control individuals were identical to those in a previous study (Nemoto-Hasebe *et al.*, 2010). In this AE cohort, 73 AE patients (mean age, 25.4 ± 8.9 years) experienced complications with asthma. Furthermore, we studied another Japanese asthma cohort (137 patients; mean age, 58.2 ± 16.9 years). Patients were considered asthmatic based on the presence of recurrent episodes of ≥ 2 of the three symptoms (coughing, wheezing, or dyspnea) associated with demonstrable reversible airflow limitation, either spontaneously or with an inhaled short-acting β_2 -agonist and/or increased airway responsiveness to methacholine (Isada *et al.*, 2010). Fully informed consent was obtained from the participants or their legal guardians for this

study. This study had been approved by the Ethical Committee at Hokkaido University Graduate School of Medicine and was conducted according to the Declaration of Helsinki Principles.

FLG mutation screening revealed that 27.4% of patients in our Japanese AE complicated with asthma case series carried one or more of the eight *FLG* mutations (combined minor allele frequency of 0.151, $n=146$) (Table 1). Conversely, 26.3% of Japanese AE patients without asthma carried one or more of the eight *FLG* mutations (combined minor allele frequency of 0.147, $n=198$). The *FLG* variants are also carried by 3.7% of Japanese control individuals (combined minor allele frequency of 0.019, $n=268$). We found that all compound heterozygous mutations were present in *trans* by observing transmission or haplotype analysis (Nomura *et al.*, 2007, 2008). There is a statistically significant association between the eight *FLG* mutations and AE with asthma, and between the eight *FLG* mutations and AE without asthma (Table 1). Moreover, AE complicated with asthma manifested in heterozygous carriers of *FLG* mutations with an odds ratio for AE and asthma of 9.74 (95% confidence interval 3.47–27.32), suggesting a relationship between *FLG* mutations and AE with asthma.

In the Japanese general asthma cohort, 8.0% of the asthma patients carried one or more of the eight *FLG* mutations (combined minor allele frequency of 0.04, $n=274$) (Table 2). Whereas, of the Japanese patients with asthma complicated by AE, 22.2% carried one or more of the *FLG* mutations (combined minor allele frequency of 0.11, $n=36$). In contrast, 5.9% of asthma patients without AE carried one or more of the *FLG* mutations

(combined minor allele frequency of 0.03, $n=238$). There was a statistically significant association between the eight *FLG* mutations and asthma with AE (Table 2). There was no statistically significant association between the *FLG* mutations and entire asthma patients, nor between *FLG* mutations and asthma without AE. We cannot exclude the possibility that this lack of significant association is due to the small number of the patients included in this study. We used the same control set for both case-controlled studies. Thus, strictly speaking, there is no independent replication for the control group.

Recent meta-analysis revealed that *FLG* mutations are significantly associated with asthma in the European population and there are especially, strong effects observed for *FLG* mutations for the compound phenotype, asthma in addition to eczema (Rodríguez *et al.*, 2009; van den Oord and Sheikh, 2010). In contrast, there appeared to be no association of *FLG* mutations with asthma in the absence of eczema (Rodríguez *et al.*, 2009; van den Oord and Sheikh, 2010).

This Japanese cohort has a completely different *FLG* mutation spectrum from those in the European and the North American populations. However, our results clearly confirm the strong association of *FLG* mutations with our Japanese cohort of AE patients with asthma complications, and the association of *FLG* mutations and asthma patients with AE complications, for the first time outside Europe or North America. Conversely, this study showed no significant correlation between general asthma patients and *FLG* mutations, suggesting that atopic asthma patients associated with *FLG* mutations are a minority among general asthma patients. The frequency of heterozygous, compound heterozygous, and homozygous *FLG* mutation carriers

Table 1. Atopic eczema case-control association analysis for *FLG* null variants in Japan

Genotype	R501X		3321delA		S1695X		Q1701X		S2554X		S2889X		S3296X		K4022X		Combined			
	Con	AE	Con	AE	Con	AE	Con	AE	Con	AE	Con	AE	Con	AE	Con	AE	Con	AE (total)	AE (asthma+)	AE (asthma-)
AA	134	172	133	163	133	172	134	169	133	162	132	152	134	166	134	169	129	126	53	73
Aa	0	0	1	9	1	0	0	3	1	10	2	20	0	6	0	3	5	41	18	23
aa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5 ¹	2	3
Total	134	172	134	172	134	172	134	172	134	172	134	172	134	172	134	172	134	172	73	99

Abbreviations: AE, atopic eczema; CI, confidence interval; Con, healthy control; OR, odds ratio.
For combined genotype: AE+asthma, exact *P*-value of Pearson χ^2 -test=1.909 $\times 10^{-6}$, OR and 95% CI for dominant models (AA vs aX)=9.737 (3.473–27.322); AE–asthma, exact *P*-value of Pearson χ^2 -test=7.189 $\times 10^{-7}$, OR and 95% CI for dominant models (AA vs aX)=9.191 (3.383–24.938); all AE, exact *P*-value of Pearson χ^2 -test=1.189 $\times 10^{-7}$, OR and 95% CI for dominant models (AA vs aX)=9.416 (3.625–24.450).
¹All the five patients were compound heterozygotes for minor alleles.

Table 2. Asthma case-control association analysis for *FLG* null variants in Japan

Genotype	R501X		3321delA		S1695X		Q1701X		S2554X		S2889X		S3296X		K4022X		Combined			
	Con	Asthma	Con	Asthma	Con	Asthma	Con	Asthma	Con	Asthma	Con	Asthma	Con	Asthma	Con	Asthma	Con	Asthma (total)	Asthma (AE+)	Asthma (AE-)
AA	134	137	133	137	133	137	134	137	133	133	132	132	134	136	134	136	129	126	14	112
Aa	0	0	1	0	1	0	0	0	1	4	2	5	0	1	0	1	5	11	4	7
aa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	134	137	134	137	134	137	134	137	134	137	134	137	134	137	134	137	134	137	18	119

Abbreviations: AE, atopic eczema; CI, confidence interval; Con, healthy control; OR, odds ratio.
For combined genotype: asthma+AE, exact *P*-value of Pearson χ^2 -test=0.0122, OR and 95% CI for dominant models (AA vs aX)=7.3692 (1.7715–30.6748); asthma–AE, exact *P*-value of Pearson χ^2 -test=0.5563, OR and 95% CI for dominant models (AA vs aX)=1.6124 (0.4979–5.2219); all asthma, exact *P*-value of Pearson χ^2 -test=0.1968, OR and 95% CI for dominant models (AA vs aX)=2.2523 (0.7609–6.6667).

observed in our Japanese controls was only 3.7%, which was much lower than that seen in European general population, where it is approximately 7.5%. This suggested that there may be further mutations yet to be discovered in the Japanese. As we have sequenced more than 40 Japanese families with ichthyosis vulgaris, there is now little possibility that further highly prevalent mutations will be found in the Japanese population. However, it is still possible that there might be multiple, further low-frequency *FLG* mutations discovered in the Japanese population. In addition, because of the relatively small sample size of this genetic study, further replication in association studies will be required for *FLG* mutations and asthma in Japan.

In our cohorts, serum IgE levels were extremely high (median, 3141.9 IU ml⁻¹; 25th–75th percentiles, 1276.0–9753.0 IU ml⁻¹) in AE patients with asthma (*n* = 73) in the AE cohort, compared with that in total asthma patients (median,

156.0 IU ml⁻¹; 25th–75th percentiles, 71.05–441.45 IU ml⁻¹, *n* = 137) in the asthma cohort. These findings suggest that extrinsic allergic sensitization might have an important role in atopic asthma pathogenesis. Recent studies hypothesized skin barrier defects caused by *FLG* mutation(s) allow allergens to penetrate the skin, resulting in initiation of further immune response and leading to the development of systemic allergies, including atopic asthma (Fallon *et al.*, 2009). In patients with asthma that also harbor *FLG* mutations, we could not exclude the possibility that the systemic effects of early eczema might simply influence airway responsiveness (Henderson *et al.*, 2008).

CONFLICT OF INTEREST

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

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REFERENCES

- Fallon PG, Sasaki T, Sandilands A et al. (2009) A homozygous frameshift mutation in the mouse *Flg* gene facilitates enhanced percutaneous allergen priming. *Nat Genet* 41: 602–8
- Hanifin JM, Rajka G (1980) Diagnostic features of atopic dermatitis. *Acta Derm Venereol* 92:44–7
- Henderson J, Northstone K, Lee SP et al. (2008) The burden of disease associated with filaggrin mutations: a population-based, longitudinal birth cohort study. *J Allergy Clin Immunol* 121:872–7
- Isada A, Konno S, Hizawa N et al. (2010) A functional polymorphism (-603A → G) in the tissue factor gene promoter is associated with adult-onset asthma. *J Hum Genet* 55: 167–74
- Nemoto-Hasebe I, Akiyama M, Nomura T et al. (2010) *FLG* mutation p.Lys4021X in the C-terminal imperfect filaggrin repeat in Japanese atopic eczema patients. *Br J Dermatol* 161:1387–90
- Nomura T, Akiyama M, Sandilands A et al. (2008) Specific filaggrin mutations cause ichthyosis vulgaris and are significantly associated with atopic dermatitis in Japan. *J Invest Dermatol* 128:1436–41
- Nomura T, Sandilands A, Akiyama M et al. (2007) Unique mutations in the filaggrin gene in Japanese patients with ichthyosis vulgaris and atopic dermatitis. *J Allergy Clin Immunol* 119:434–40
- Rodríguez E, Baurecht H, Herberich E et al. (2009) Meta-analysis of filaggrin polymorphisms in eczema and asthma: robust risk factors in atopic disease. *J Allergy Clin Immunol* 123:1361–70
- Sandilands A, Terron-Kwiatkowski A, Hull PR et al. (2007) Comprehensive analysis of the gene encoding filaggrin uncovers prevalent and rare mutations in ichthyosis vulgaris and atopic eczema. *Nat Genet* 39:650–4
- van den Oord RA, Sheikh A (2010) Filaggrin gene defects and risk of developing allergic sensitisation and allergic disorders: systematic review and meta-analysis. *BMJ* 339:b2433

See related commentary on pg 2703

RNase 7 Protects Healthy Skin from *Staphylococcus aureus* Colonization

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TO THE EDITOR

The Gram-positive bacterium *Staphylococcus aureus* is an important pathogen that causes various skin infections (Miller and Kaplan, 2009). However, healthy skin is usually not infected by *S. aureus*, despite the high carrier rates in the normal population (Noble, 1998). This suggests that the cutaneous defense system has the capacity to effectively control the growth of *S. aureus*. There is increasing evidence that antimicrobial proteins are important effectors of the cutaneous defense system (Harder et al., 2007). A recent study reported that keratinocytes contribute to cutaneous innate defense against *S. aureus* through the production of human β -defensin-3 (Kisich et al., 2007). In addition to human β -defensin-3, other antimicrobial proteins may also participate in cutaneous defense against *S. aureus*. One candidate is RNase 7, a potent antimicrobial ribonuclease that is highly expressed in healthy skin (Harder and Schröder, 2002; Köten et al., 2009).

To investigate the hypothesis that RNase 7 may contribute to protect

healthy skin from *S. aureus* colonization, we first incubated natural RNase 7 isolated from stratum corneum skin extracts (Harder and Schröder, 2002) with *S. aureus* (ATCC 6538). In concordance with our initial report about RNase 7 (Harder and Schröder, 2002), we verified that RNase 7 exhibited

a high killing activity against *S. aureus* (lethal dose of 90% = 3–6 $\mu\text{g ml}^{-1}$).

Recently, we reported a moderate induction of RNase 7 mRNA expression in primary keratinocytes treated with heat-killed *S. aureus* (Harder and Schröder, 2002). To assess the induction of RNase 7 by *S. aureus* in the

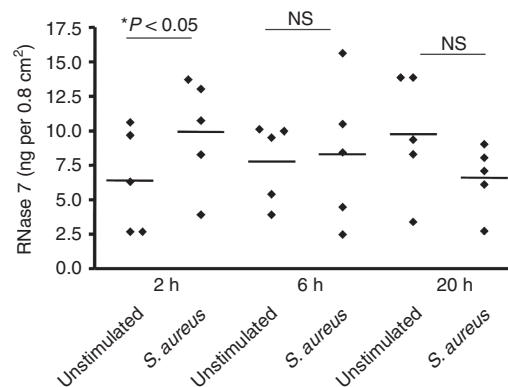


Figure 1. Induced secretion of RNase 7 on the skin surface on treatment with living *S. aureus*.

Defined areas (0.8 cm²) of skin explants derived from plastic surgery were incubated with or without approximately 1,000 colony-forming units of *S. aureus* (ATCC 6538) in 100 μl of sodium phosphate buffer. After 2, 6, and 20 hours, the concentration of secreted RNase 7 was determined by ELISA. Stimulation with *S. aureus* for 2 hours revealed a significant induction as compared with the unstimulated control after 2 hours (*P < 0.05, Student's *t*-test; n.s. = not significant). Data shown are means of triplicates of five skin explants derived from five donors.