

LETTER

Filaggrin mutations are not associated with chronic allergic keratoconjunctivitis

Filaggrin gene mutations are predisposing genetic factors for atopic dermatitis (AD).¹ In this study, we investigated the association between atopic keratoconjunctivitis (AKC)/vernal keratoconjunctivitis (VKC) and filaggrin gene mutations using a Japanese population. Detailed information on the diagnostic criteria for AKC and VKC was provided elsewhere.² The mean age of VKC patients was 15.7 ± 8.9 years (7–32 years), that of AKC patients 28.34 ± 9.9 years (6–50 years) and for controls 42.7 ± 24.3 years (23–67 years). The male:female ratios were 3.2:1.0 for VKC, 3.9:1.0 for AKC and 1:1 for controls.

Four common filaggrin gene mutations in the Japanese population (S2554X, S2889X, S3296X, 3321delA)³ were genotyped using DNA samples obtained from 69 cases (AKC 44; VKC 25) enrolled in this study. Ninety-three DNA samples obtained from healthy controls (from Health Science Research Resources Japan, Osaka, Japan) were also genotyped. Direct sequence analysis was carried out by dye-terminator methods. Filaggrin gene messenger RNA expression in cultured conjunctival epithelial cells⁴ was examined by reverse transcription PCR. Total RNA was extracted and reverse transcription was carried out using random hexamers. We designed PCR primers at the boundary region of exons 2 and 3 of the filaggrin gene (forward 5'-TGCCATAATTAATCTTTTCAAGCA-3', reverse 5'-TGCTTTCTGTGCTTGTGTCC-3').

For immunohistological analysis, the giant papillae (n=4) were fixed with 4% paraformaldehyde and paraffin sections were made. A mouse anti-filaggrin gene monoclonal antibody (clone SPM182) purchased from AnaSpec, Inc (San Jose, California, USA) and mouse control IgG1 from Dako Japan (Kyoto, Japan) were used as primary antibodies. Biotin-conjugated goat anti-mouse IgG (Dako) was used as the secondary antibody and then, after incubation with streptavidin-peroxidase, the positive filaggrin gene signals were visualised using

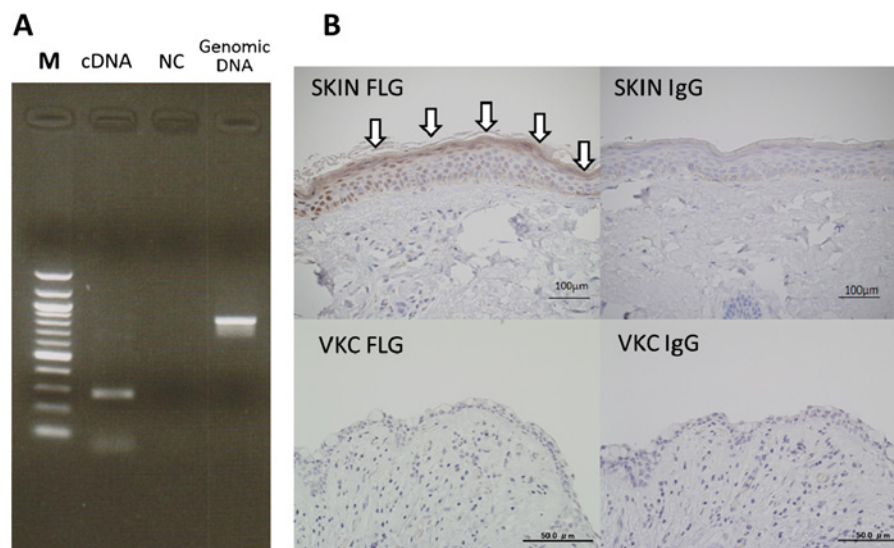


Figure 1 (A) Reverse transcription (RT) PCR analysis of filaggrin gene (FLG) mRNA expression in cultured human conjunctival cells (HCjE). RT-PCR was performed with HCjE complementary DNA, negative control (NC; HCjE sample without reverse transcriptase) and genomic DNA from HCjE. Filaggrin gene mRNA yielded a band at 285 bp; the genomic filaggrin gene sequence yielded a band at 870 bp. M, 100 bp DNA ladder marker. (B) Immunohistochemical analysis of normal eyelid skin (SKIN) and giant papilla tissue from a vernal keratoconjunctivitis (VKC) patient. Positive filaggrin gene immunostaining was observed in the cornified layer of the skin (arrows, FLG SKIN). No positive filaggrin gene staining was observed in VKC samples (FLG VKC). Negative control staining with control IgG is also shown (SKIN IgG and VKC IgG).

3,3'-diaminobenzidine, followed by counterstaining with haematoxylin. Normal skin tissues from eyelids (n=4) obtained during ptosis surgery were also stained as a positive control. All procedures were approved by the ethics committees of Juntendo University and Kyoto Prefectural University of Medicine, and this study was conducted in accordance with the tenets of the Declaration of Helsinki.

Our results revealed no significant association between filaggrin gene mutations and chronic allergic keratoconjunctivitis (table 1). The two patients with the S2554X mutation both had AKC. The patient with the S3296X mutation had VKC. We also carried out statistical comparison between filaggrin gene mutations and AKC (chronic allergic keratoconjunctivitis with AD), but found no association.

As the filaggrin gene mutation spectrum is completely different from those in the Caucasian population, we genotyped the four most common filaggrin gene mutations among

Japanese.³ We also tried to clarify filaggrin gene mRNA and protein expression using conjunctival epithelial cells and tissue. Although we detected filaggrin gene mRNA expression in conjunctival epithelial cells (figure 1A), no filaggrin gene protein expression was observed in conjunctival tissue by immunohistochemistry (figure 1B). These results are consistent with the results of Ying *et al*,⁵ in which positive filaggrin gene protein expression was observed in human skin but not in bronchial epithelium, and of Nakamura *et al*⁶ showing filaggrin gene protein expression in cicatrising ocular surface diseases but not in normal conjunctivae. The negative association between filaggrin gene mutations and chronic allergic conjunctivitis and lack of filaggrin gene protein expression in VKC/AKC suggested that filaggrin gene mutations did not exert organ-specific effects on the pathophysiology of allergic conjunctivitis in the ocular surface. These results are consistent with the results of Osawa *et al*,³ which showed genetic association between

Table 1 Genotyping of filaggrin gene mutation for chronic allergic conjunctivitis

Genotype	S2554X		S3296X		S2889X		3321delA	
	control	AKC/VKC	control	AKC/VKC	control	AKC/VKC	control	AKC/VKC
AA	89	67	93	68	93	69	93	69
Aa	4	2	0	1	0	0	0	0
aa	0	0	0	0	0	0	0	0
Totals	93	69	93	69	93	69	93	69
χ^2	p=0.64		p=0.24		NA		NA	

p, p Value of χ^2 test for dominant models (AA vs Aa + aa).

The two patients with the S2554X mutation were atopic keratoconjunctivitis (AKC) patients, and the patient with the S3296X mutation was a vernal keratoconjunctivitis (VKC) patient. NA, not applicable.

filaggrin gene mutations and asthma with AD but not for asthma without AD.

In summary, we could not detect a positive association between filaggrin gene mutations and chronic allergic keratoconjunctivitis in the Japanese study population. Due to the small size of this genetic association study, replication studies using both Oriental and Caucasian populations will be essential to draw firm conclusions.

Acknowledgements The authors would like to thank Professor Ilene K Gipson for providing human conjunctival epithelial cells and Professor Shigeru Kinoshita for clinical sample collection.

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Contributors SI, KH and YA performed experiments. NE, TF, NY, TI and SK collected clinical samples and analysed the data. AMu obtained the grant and wrote the paper. AMa planned the experiments, analysed the data, obtained the grant and wrote the paper.

Funding This study was supported by grants-in-aid from MEXT Japan (nos 21592239 and 24592652 to AMa, and no 24659768 to AMu).

Competing interests None.

Ethics approval Ethics approval was provided by Juntendo University and Kyoto Prefectural University.

Provenance and peer review Not commissioned; internally peer reviewed.

Br J Ophthalmol 2012;■:1–2.
doi:10.1136/bjophthalmol-2012-301990

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Br J Ophthalmol published online May 26, 2012

doi: 10.1136/bjophthalmol-2012-301990

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Published online May 26, 2012 in advance of the print journal.

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