Short Communication

Filaggrin Gene Mutation c.3321delA is Associated with Dry Phenotypes of Atopic Dermatitis in the Chinese Han Population

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Introduction

Atopic dermatitis (AD) is a common chronic inflammatory skin disorder that is characterized by dry skin and disturbed skin barrier functions. Mutations in the filaggrin (*FLG*) gene, the gene coding profilaggrin/filaggrin, have a great impact on the epidermal barrier function and are an important predisposing factor for AD. However, in both Europeans and Asians, the presence of *FLG* mutations has been reported to be population-specific. *FLG* mutation c.3321delA is an Asian-specific mutation and is the most common mutation in the Chinese population, accounting for up to 15% of patients with AD.[1] In a previous genome-wide association study of AD, Meng *et al*.[2] identified *FLG* variant rs3126085, which was correlated with c.3321delA. Several studies have shown that the c.3321delA mutation was associated with various AD-associated phenotypes, most of which are related to dry skin phenotypes, including xerosis, ichthyosis vulgaris (IV), and palmar hyperlinearity.[2] In this study, we used high-resolution melting analysis (HRMA) with unlabeled probe for the detection of c.3321delA mutation in Chinese AD patients and investigated the genotype-phenotype correlation between c.3321delA and atopic characteristics.

Methods

Participants

A total of 547 AD patients (276 men and 271 women, mean age: 13.0 ± 12.0 years) and 470 controls (201 men and 269 women, mean age: 11.7 ± 4.3 years) of Han Chinese ethnicity were enrolled. The patients were diagnosed with AD according to the Hanifin and Rajka diagnostic criteria. Demographic and clinical information were collected as shown in Table 1, including gender, disease onset time, and personal history of atopic disorders. The following four dry skin phenotypes, including xerosis, IV, palmar hyperlinearity, and keratosis pilaris, were scored as 0 (not present) or 1 (present). A global clinical dry skin score was determined as the sum of the scores of each dry skin phenotype (0–4). All controls were clinically assessed to be without atopic diseases, a family history of atopic diseases (including first-, second-, and third-degree relatives) or IV. In this study, we used the HRMA with unlabeled probe (this method in detail has been described in our previous study[3])to detect the c.3321delA mutation in all participants through a rapid, cost-efficient and reliable closed-tube method compared with other genotyping methods, including DNA sequencing and restriction fragment length polymorphism. The study was approved by the Ethical Committee of Peking University

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Shenzhen Hospital and was conducted according to the *Declaration of Helsinki* principles. Written informed consent was obtained from all participants.

Statistical analysis

Statistical analysis was performed using SPSS (version 13.0) statistical software (SPSS Inc., Chicago, IL, USA). The allele frequencies and genotype distributions were calculated using the gene-counting method. The Chi-square exact test was used to evaluate the allelic and genotypic frequencies and to estimate the Hardy-Weinberg equilibrium. The value of $P < 0.05$ was considered to indicate a statistically significant difference.

Results

With unlabeled probe based HRMA, we successfully genotyped the 1017 blood samples. Genotype frequencies in the patients and controls were in Hardy-Weinberg equilibrium.

As shown in Table 2, statistically significant differences were observed in both genotype and allele frequencies for c.3321delA mutation in patients with AD compared with controls. The prevalence of the c.3321delA mutation in Chinese patients with AD was 7.13% compared with 3.19% in healthy controls ($P = 0.009$). The allele A deletion was found to be a risk factor for AD ($P = 0.004$, odds ratio [*OR*] = 2.340, 95% confidence interval [*CI*]: 1.284–4.264).

The association between c.3321delA mutation and AD-associated phenotypes was assessed [Table 1]. Significant associations were found between c.3321delA and some AD-associated phenotypes including IV ($P = 0.006$, $OR = 2.393$, 95% *CI*: 1.269–4.511) and palmar hyperlinearity ($P = 0.022$, $OR = 2.064$, 95% *CI*: 1.096–3.887). For four AD-associated dry phenotypes, further analysis showed that AD patients with global clinical dry skin scores ≥2 were significantly

Table 1: The association between c.3321delA mutation and clinical phenotypes in atopic dermatitis (*N* = 547)

Clinical phenotypes	AD, *n* (%)	Allele frequencies		χ^2	*P*
		ΔA	A		
Early age of onset (≤2 years)	288 (52.65)	4.17	95.83	0.899	0.343
Early age of onset (>2 years)	259 (47.35)	3.09	96.91		
AD with asthma	47 (8.59)	3.19	96.81	0.000	1.000
AD without asthma	500 (91.41)	3.70	96.30		
AD with allergic rhinitis	237 (43.33)	3.59	96.41	0.012	0.914
AD without allergic rhinitis	310 (56.67)	3.71	96.29		
AD with infraorbital fold	164 (29.98)	3.05	96.95	0.491	0.484
AD without infraorbital fold	383 (70.02)	3.92	96.08		
AD with orbital darkening	185 (33.82)	3.78	96.22	0.026	0.872
AD without orbital darkening	362 (66.18)	3.59	96.41		
AD with white dermatographism	20 (3.66)	5.00	95.00	0.001	0.974
AD without white dermatographism	527 (96.34)	3.61	96.39		
AD with perifollicular accentuation	130 (23.77)	3.08	96.92	0.325	0.569
AD without perifollicular accentuation	417 (76.23)	3.84	96.16		
AD with xerosis	492 (89.95)	3.66	96.34	0.000	1.000
AD without xerosis	55 (10.05)	3.64	96.36		
AD with IV	177 (32.36)	5.93	94.07	7.695	0.006
AD without IV	370 (67.64)	2.57	97.43		
AD with palmar hyperlinearity	182 (33.27)	5.49	94.51	5.233	0.022
AD without palmar hyperlinearity	365 (66.73)	2.74	97.26		
AD with keratosis pilaris	78 (14.26)	1.92	98.08	1.155	0.213
AD without keratosis pilaris	469 (85.74)	3.94	96.06		
AD with global score* (≥2)	221 (40.40)	5.80	94.12	10.433	0.001
AD with global score (<2)	326 (59.60)	2.15	97.85		

*Four dry skin phenotypes were scored as 0 (not present) or 1 (present): xerosis, IV, palmar hyperlinearity, and keratosis pilaris; and a global clinical dry skin score was determined as the sum of score of each dry skin phenotype (0–4). AD: Atopic dermatitis; IV: Ichthyosis vulgaris; *OR*: Odds ratio; *CI*: Confidence interval; ΔA: c.3321delA.

Table 2: The association of atopic dermatitis with c.3321delA mutation

Population	Genotype frequency, *n* (%)					Allele frequency, *n* (%)			
	AA	A/ΔA	ΔA/ΔA	χ^2	*P*	ΔA	A	χ^2	*P*
AD (*n* = 547)	508 (92.87)	38 (6.95)	1 (0.18)	8.156	0.009	40 (3.66)	1054 (96.34)	8.159	0.004
Controls (*n* = 470)	455 (96.81)	15 (3.19)	0			15 (1.60)	925 (98.40)		

AD: Atopic dermatitis; *OR*: Odds ratio; 95% *CI*: 95% confidence interval; ΔA: c.3321delA.

associated with c.3321delA ($P = 0.001$, $OR = 2.848$, 95% *CI*: 1.470–5.518). There was no significant difference between c.3321delA and other AD-associated phenotypes, including gender, family history of atopic disorders (data not shown), early age of onset, infraorbital fold, orbital darkening, white dermatographism, and perifollicular accentuation ($P > 0.05$) [Table 1].

Discussion

FLG mutation c.3321delA, first identified in the Japanese population, is an Asian-specific mutation that has been reported in Chinese, Korean, and Singaporean populations.[2] Our findings showed that c.3321delA was significantly associated with AD, which was consistent with several other Chinese case-control studies and a family association study. However, in this study, the prevalence of c.3321delA mutation carriers in 547 cases was only 7.13%, which was much lower than that reported by other Chinese studies (from 9.7% to 15%).[1,4] *FLG* mutations have been shown to display ethnic and/or geographic specificity, and the mutation pattern in studied Asian populations, especially in the Chinese population, is even more distinct and complex. Therefore, the lower mutation rate presented in this study may be due to the regional difference or the smaller sample size.

We also found significant associations between c.3321delA and two AD-related dry skin phenotypes (IV and palmar hyperlinearity). These findings were consistent with previous studies regarding *FLG* compound mutations and were consistent with the largest sample size correlation study between single c.3321delA mutations and AD phenotypes.[2] However, in this study, no association between xerosis, keratosis pilaris and c.3321delA were observed, which was inconsistent with the study employed by Meng *et al.*,[2] but was consistent with a recent study in northern China.[4] This might be due to the high percentage of AD patients also having xerosis [approximately 90%, Table 1] and the lower c.3321delA mutation rate. However, a significant association was found between c.3321delA and AD patients with global clinical dry skin scores ≥2. All four dry phenotypes are related to skin barrier dysfunction in AD patients.[2] Therefore, the global clinical dry skin scores might represent good phenotypic indicators of *FLG* c.3321delA mutation in Chinese patients with AD.

Ezzedine *et al.*[5] derived a global IV clinical severity score (0–15) by scoring each of five IV clinical signs (diffuse xerosis, hyperlinearity of palms, scales on legs, scalp desquamation, and keratosis pilaris) from 0 to 3 points and genotyping two common *FLG* null mutations (R501X and 2282del4) for 110 Caucasian patients. They found out that the global clinical severity scores and 2282del4 mutations were positively correlated with the AD concomitant IV phenotype. Thus, they reported a useful global clinical IV scoring system for predicting common *FLG* null mutations in an adult Caucasian population.

In conclusion, mutation c.3321delA was associated with Chinese AD, and the AD-associated dry skin phenotypes might be good phenotypic indicators of *FLG* c.3321delA mutations in the Chinese AD patients.

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Conflicts of interest

There are no conflicts of interest.

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