Filaggrin Gene Mutation c.3321delA Is Associated with Various Clinical Features of Atopic Dermatitis in the Chinese Han Population

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Abstract

Background: We confirmed that the filaggrin gene mutation c.3321delA is associated with atopic dermatitis in our previous genome wide association study of the Chinese Han population. c.3321delA is the most common filaggrin gene mutation in Chinese atopic dermatitis patients but is not present in European populations.

Objective: To investigate the genetic model for the c.3321delA mutation and to determine the correlation between c.3321delA and atopic dermatitis clinical phenotypes in the Chinese Han population.

Method: The filaggrin gene mutation c.3321delA was sequenced in 1,080 atopic dermatitis patients and 908 controls from the Chinese population. The χ² test, ANOVA, non-parametric tests and logistic regression were used to investigate the relationship between the c.3321delA genotype and atopic dermatitis clinical phenotypes in the Chinese Han population.

Results: Analyses of the genetic model revealed that the additive model best described the c.3321delA mutation (P = 3.09E-11, OR = 3.43, 95% CI = 2.38–4.96). Stratified analyses showed that the c.3321delA allele frequency distribution is significantly associated with concomitant skin xerosis (P = 1.68E-03, OR = 2.13, 95% CI = 1.32–3.46), palmar hyperlinearity (P = 3.64E-17, OR = 4.0, 95% CI = 2.86–5.70), white dermatographism (P = 4.25E-03, OR = 1.82, 95% CI = 1.22–2.71), food intolerance (P = 1.51E-03, OR = 1.76, 95% CI = 1.23–2.50) and disease severity (P = 9.67E-05).

Conclusion: Our study indicates that the filaggrin gene mutation c.3321delA is associated with clinical phenotypes of atopic dermatitis in the Chinese Han population, which might help us gain a better understanding on the pathogenesis of atopic dermatitis.

Introduction

Atopic dermatitis (AD) has long been recognized as a complex trait, wherein multiple genes and environmental stimuli contribute to disease manifestation. To date, 81 genes have been implicated in over 100 published reports on AD genetic association studies, and 46 of these genes have demonstrated at least one positive association with AD. Of these genes, filaggrin gene (FLG) is the most consistently replicated gene, appearing in 20 reports [1]. Filaggrin, also known as filament-aggregating protein, plays a major role in the epidermal barrier function. To date, approximately 60 loss-of-function FLG mutations have been identified in European and Asian populations [2]. All of the mutations that are predicted to cause loss of function, including nonsense mutations as well as out-of-frame insertions or deletions, are specific to certain ethnic groups, with distinct profiles observed in the European and Asian populations that have been well studied [3].
AD, with a prevalence of 1.4 to 22.3% worldwide [4], has a complex clinical phenotype strongly associated with food allergies, asthma, and allergic rhinitis (AR) in a patient’s life (i.e., the atopic march) [5]. Over the last decade, numerous association studies on FLG mutations and AD-associated phenotypes have been conducted. The majority of the studies focused on combined FLG mutations, and only a few studies referred to single mutation. c.3321delA is an Asian-specific FLG mutation that has been described in Chinese, Japanese, Korean and Singaporean populations [6]. c.3321delA is the most common FLG mutation in the Chinese population; however, it is not found in European populations. In our previous Genome Wide Association Study (GWAS) of AD, we identified the FLG variant rs3126005 that correlates with c.3321delA. In this study, we investigated the genetic model for c.3321delA, the genotype–phenotype correlation between c.3321delA and AD in the Chinese population. This study employs the largest sample size of all of the genotype-phenotype correlation studies of AD to date.

Materials and Methods

Patients and controls

A total of 1,080 AD patients and 908 controls were enrolled in this study (Table 1). All samples were from the Chinese Han population and were used in our previous GWAS. AD patients meeting the Hanifin-Rajka diagnostic criteria [7] were recruited from the No. 1 Hospital of Anhui Medical University and the Xinhua Hospital affiliated with the Shanghai Jiaotong University School of Medicine in China. Physician specialists collected clinical data from the affected individuals through a full clinical checkup. Additional demographic information was collected from the cases and controls through a structured questionnaire. The disease severity was evaluated using the objective SCORing Atopic Dermatitis (SCORAD) index [8], which categorizes patients as mild (≤15 points), moderate (15–40 points) and severe (>40 points). Patients were considered to have food intolerance either evaluated by allergen test of venous blood samples or patients’ self-reports. All controls were clinically assessed to be without AD or other atopic diseases, a family history of atopic diseases (including first-, second- and third-degree relatives) or ichthyosis vulgaris (IV).

All participants provided written informed consent. The study was approved by the Institutional Ethics Committee of Anhui Medical University and was conducted according to the Declaration of Helsinki principles.

Statistical analyses

c.3321delA genotyping adhered to quality control standards, with a call rate >95% and meeting Hardy–Weinberg equilibrium (P>0.01) in the controls. The c.3321delA allele and genotypic frequencies were compared between the AD patients and controls using the χ² test with 2x2 and 2x3 contingency tables (SPSS 10.0, Statistical Program for Social Sciences, Illinois). The Fisher’s exact test was used to compare the variable frequencies when the expected count was less than 5. Stratified analyses were performed to examine the relation between c.3321delA and the AD phenotypes. P<0.05 (two-tailed) was considered significant. The genetic models (dominant, recessive and additive models) were calculated for c.3321delA using logistic regression. To assess the effect of c.3321delA on the age of onset and disease severity, quantitative trait locus (QTL) analyses were performed in cases using ANOVA and nonparametric tests.

Results

Characteristics of the study subjects

The clinical characteristics of 1,080 patients (629 male and 451 female with a mean age of 5.14±6.42 years), including age of onset, AD with asthma, AD with AR, total IgE and AD severity, are summarized in Table 1. The 908 controls (629 male and 451 female) have a mean age of 16.35±9.32 years.

The association of AD with c.3321delA

The c.3321delA FLG mutation was significantly associated with AD (P = 3.09 E-12, OR = 3.43, 95% CI = 2.38–4.96; Pgenotype = 1.75E-11, OR = 2.93, 95% CI = 2.00–4.28). We further evaluated the homozygous and heterozygous odds ratio (ORhom/ ORhet) for c.3321delA in the cases and controls. Using allele A as the reference allele, the ORhet estimate of c.3321delA was 2.93 (95% CI = 2.00–4.28); however, the ORhom estimates could not be calculated because no controls were homozygous for c.3321delA (Table 2). Overall, the genetic model analysis revealed that the additive model best described the association of c.3321delA with AD (P = 3.09E-11, OR = 3.43, 95% CI = 2.38–4.96).

Clinical phenotype stratification analyses

We also assessed the association between c.3321delA and AD phenotypes (Table 3) using stratified analyses in the cases. Significant associations were observed between c.3321delA and concomitant skin xerosis (P = 1.68E-03, OR = 2.13, 95% CI = 1.32–3.46), IV (P = 2.17E-02, OR = 1.63, 95% CI = 1.07–2.49), palmar hyperlinearity (P = 3.64E-17, OR = 4.03, 95% CI = 2.36–7.50), keratosis pilaris (P = 1.72E-02, OR = 1.70, 95% CI = 1.09–2.64), white dermatographism (P = 4.25E-03, OR = 1.82, 95% CI = 1.22–2.71) and food intolerance (P = 1.51E-03, OR = 1.76, 95% CI = 1.23–2.50) (Table 3). In the QTL analysis, we found that c.3321delA was associated with disease severity (P = 9.67E-05). The c.3321delA homozygous and heterozygous patients displayed a significantly increased average SCORAD score (32.87 and 30.79, respectively) compared with the patients with a wild-type genotype (25.73) (Table 4). The patients harboring c.3321delA (homozygous and heterozygous) displayed a trend of earlier age of onset (0.16 and 0.81 years, respectively) compared with the wild-type genotype (1.07 years), which although displayed no statistical significance but showed a trend among three groups (P = 0.056) (Table 4). We observed that the c.3321delA allele frequencies in AD patients without asthma or AR were slightly higher than in patients with asthma or AR, but the differences were not statistically significant (all P>0.05) (Table 3). In stratified analyses, c.3321delA was not associated with other phenotypes of AD, including early age of onset, elevated total serum IgE levels and orbital darkening (P>0.05) (Table 3).

The relationship between age of onset and c.3321delA-associated phenotypes of AD

In order to explore whether patients with earlier onset tend to present the phenotypes associated with c.3321delA or whether patients with mutation related phenotypes display a trend of earlier onset, we divided the patients into two groups (early age of onset and late age of onset), calculated these phenotypes’ prevalence of the two groups, and compared the phenotype-distribution difference using the χ² test. We observed that the prevalence of AD concomitant with IV in the early age of onset group was significant lower than late age of onset group (13.37% vs 27.27%, P = 0.004). On the other hand, AD concomitant with IV displayed a trend of later onset than without IV (1.56 years vs 0.91 years, P = 0.026), as well as AD concomitant with keratosis.
Pilaris also had a trend of later onset than without keratosis pilaris (1.69 years vs 0.91 years, \( P = 0.034 \)) (Table 5). There was no significant difference between age of onset and phenotypes (xerosis, palmar hyperlinearity, food intolerance and white dermatographism) (Table 5).

**Discussion**

In our previous GWAS, we confirmed that the FLG mutation c.3321delA is associated with AD in the Chinese Han population [9]. In the current study, our genotype-phenotype analyses of AD may aid in the investigation of various disease phenotypes and the identification of phenotype-specific genetic factors, thereby providing new insights into the pathogenesis of AD. Our findings indicate that c.3321delA significantly associates with various AD clinical phenotypes, including skin xerosis, IV, palmar hyperlinearity, keratosis pilaris, white dermatographism, food intolerance and disease severity.

The association of c.3321delA in our group was best described with an additive model that displayed a clear trend for increased disease risk in heterozygous and homozygous c.3321delA patients. Our analysis comparing AD severity (measured by the objective SCORAD score) between the genotype groups in the QTL analysis showed that homozygous and heterozygous c.3321delA patients were more likely to have a more severe form of the disease, and this finding is consistent with the Singaporean study that showed the combined null FLG genotype of 17 mutations detected in cases and controls were strongly associated with increased AD severity (permutation test \( P = 0.0063 \)) [6]. However, the association was inconsistent in other studies [2,10,11] in the Chinese Han population, all of which were assessing compound genotypes of FLG including c.3321delA with smaller samples.

### Table 1. The clinical characteristics of 1,080 cases.

| Phenotype                          | Patients          |
|-----------------------------------|-------------------|
| Male (%)                          | 629(58.24%)       |
| Female (%)                        | 451(41.76%)       |
| Age (years), mean±SD (range)      | 5.14±6.42(0.5–58) |
| Age of onset (years), mean±SD (range) | 1.03±3.00(0.02–37) |
| Early age of onset (≤2 years) (%) (n*) | 999(92.59%)(1,079) |
| AD with asthma (%) (n*)           | 246(22.82%)(1,078) |
| AD with allergic rhinitis (%) (n*) | 344(32.12%)(1,071) |
| AD with xerosis (%) (n*)          | 812(75.19%)(1,080) |
| AD with IV (%) (n*)               | 155(14.35%)(1,080) |
| AD with palmar hyperlinearity (%) (n*) | 237(22.11%)(1,072) |
| AD with keratosis pilaris (%) (n*) | 134(12.51%)(1,071) |
| AD with orbital darkening (%) (n*) | 89(8.27%)(1,076)   |
| AD with food intolerance (%) (n*)  | 407(42.66%)(954)   |
| AD with white dermatographism (%) (n*) | 158(14.65%)(1,080) |
| Elevated total IgE (>100 IU/ml) (%) (n*) | 575(66.45%)(816)   |
| Mild AD (objective SCORAD≤15) (%) (n*) | 179(16.57%)(1,080) |
| Moderate AD (15< objective SCORAD≤40) (%) (n*) | 764(70.74%)(1,080) |
| Severe AD (objective SCORAD>40) (%) (n*) | 137(12.69%)(1,080) |

**Table 2. Genotype of c.3321delA in 1,080 cases and 908 control.**

| Genotype  | Cases (n = 1080) | Controls (n = 908) | OR (95% CI) | P   |
|-----------|-----------------|--------------------|-------------|-----|
| AA        | 949(87.87%)     | 871(95.93%)        | Reference   |     |
| Aa        | 118(10.93%)     | 37(4.07%)          | 2.93(2.00–4.28) | 1.75E-11 |
| aa        | 13(1.20%)       | 0(0%)              | NA          |     |
| Recessive model |         |                    |       |     |
| aa/(Aa+AA)| 13/1067        | 0/908              | NA          | 9.10E-04 |
| Dominant model |          |                    |       |     |
| (aa+Aa)/AA| 131/949        | 37/871             | 3.25(2.23–4.73) | 1.26E-10 |
| Additive model |      |                    |       |     |
| aa/Aa/AA  | 131/118/949    | 0/37/871           | 3.43(2.38–4.96) | 3.09E-11 |
mutations predict dose-dependent alterations in epidermal permeability barrier function [12], and our results confirmed that the FLG null mutations might serve as an indicator of severe disease phenotypes.

Several studies indicate that FLG mutations have an effect on the age of onset of AD such that individuals carrying FLG mutations (R501X, 2282del4, R2447X or S3247x) can lead to early-onset (age of onset <2 years) AD that persists well into adulthood [13–16]. Moreover, Ma et al. reported that c.3321delA was associated with early-onset of AD in Northern Chinese patients (P = 0.020) [17]. However, in our study, no statistical significance was observed for the association between early-onset at AD and FLG mutation c.3321delA in stratified analysis (P = 1.24E-01), which may be attributed to the fact that the majority of AD cases begin early in life (age of onset <2 years). It’s reported that FLG mutations were associated with much earlier age at onset for AD [11,18], AD patients carrying FLG mutations were younger than those without FLG mutations. However, one study in China did not observe the association (P = 0.307) [2]. In our study, we observed that the average/median age of onset in AD tended to decrease among the three groups (wide-type, homozygous and heterozygous genotype) in the QTL analysis, but there were no statistical differences (P = 0.056), which may be due to the low proportion of AD cases with homozygous c.3321delA. Further analysis using larger sample sizes will be helpful for determining the effect of c.3321delA on age of onset.

Our data provide evidence for the association between c.3321delA and clinical phenotypes, including concomitant IV, palmar hyperlinearity, and keratosis pilaris (Table 3), consistent with previous studies [6,10] regarding FLG compound mutations. A recent study in Northern China indicated that combined FLG variants were significantly associated with IV and palmar hyperlinearity; however, no association with keratosis pilaris was observed [2]. These results are attributed to the fact that AD has a well-recognized association with IV [19,20] and that FLG is the pathogenic gene of IV; thus, AD patients from non-IV

| Table 3. The association between c.3321delA and clinical phenotypes in AD. |
|---------------------------------|----------------|-----------------|----------------|
| Clinical phenotypes            | Allele frequencies |  P        | OR  | 95%CI                           |
|---------------------------------|----------------|----------------|----------------|
|                                | 3321delA | A   |     |                                  |
| Early age of onset (<2 years)  | 0.0691 | 0.9309 | 1.24E-01 | 1.90 | 0.83–4.38 |
| Late age of onset (>2 years)   | 0.0375 | 0.9625 |         |     |          |
| AD with asthma                  | 0.0650 | 0.9350 | 8.60E-01 | 0.96 | 0.64–1.45 |
| AD without asthma               | 0.0673 | 0.9327 |         |     |          |
| AD with AR                      | 0.0581 | 0.9419 | 2.97E-01 | 0.82 | 0.56–1.19 |
| AD without AR                   | 0.0702 | 0.9299 |         |     |          |
| AD with elevated IgE            | 0.0740 | 0.9260 | 6.59E-01 | 1.10 | 0.77–1.56 |
| AD with normal IgE              | 0.0685 | 0.9315 |         |     |          |
| AD with Xerosis                 | 0.0764 | 0.9237 | 1.68E-03 | 2.13 | 1.32–3.46 |
| AD without Xerosis              | 0.0373 | 0.9627 |         |     |          |
| AD with IV                      | 0.0968 | 0.9032 | 2.17E-02 | 1.63 | 1.07–2.49 |
| AD without IV                   | 0.0616 | 0.9384 |         |     |          |
| AD with Palmar hyperlinearity   | 0.1519 | 0.8481 | 3.64E-17 | 4.03 | 2.86–5.70 |
| AD without Palmar hyperlinearity| 0.0426 | 0.9575 |         |     |          |
| AD with Keratosis pilaris       | 0.1007 | 0.8993 | 1.72E-02 | 1.70 | 1.09–2.64 |
| AD without Keratosis pilaris    | 0.0619 | 0.9381 |         |     |          |
| AD with Orbital darkening       | 0.0506 | 0.9494 | 3.62E-01 | 0.73 | 0.36–1.45 |
| AD without Orbital darkening    | 0.0684 | 0.9316 |         |     |          |
| AD with food intolerance        | 0.0842 | 0.9158 | 1.51E-03 | 1.76 | 1.23–2.50 |
| AD with food tolerance          | 0.0496 | 0.9504 |         |     |          |
| AD with White dermatographism   | 0.1107 | 0.8893 | 4.25E-03 | 1.82 | 1.22–2.71 |
| AD without White dermatographism| 0.0642 | 0.9358 |         |     |          |

doi:10.1371/journal.pone.0098235.t003

| Table 4. Association between genotype of c.3321delA and SCORAD and age of onset in AD. |
|---------------------------------|----------------|----------------|----------------|
| Genotypes                       | AA   | Aa  | aa   | P       |
| Patients (n)                    | 927  | 118 | 13   |         |
| Objective SCORAD (median)       | 25.73(24.00) | 30.79(29.30) | 32.87(32.00) | 9.67E-05 |
| Age of onset (years) (median)   | 1.07 (0.17) | 0.81 (0.17) | 0.16 (0.083) | 0.056   |

doi:10.1371/journal.pone.0098235.t004
Table 5. The relationship between age of onset and c.3321delA related phenotypes in AD.

| c.3321delA related phenotypes of AD | Early age of onset | Late age of onset | P* | OR(95%CI) | Cases(n) | Mean onset age(SD) | P* |
|-------------------------------------|-------------------|------------------|----|-----------|----------|-------------------|----|
| With xerosis                         | 750(75.53%)       | 56(72.73%)       | 0.953 | 0.99(0.69–1.42) | 812 | 0.95(3.10) | 0.256 |
| Without xerosis                       | 243(24.47%)       | 21(27.27%)       | 0.004 | 2.09(1.25–3.50) | 154 | 1.56(3.35) | 0.026 |
| With IV                              | 855(86.63%)       | 56(72.73%)       | 0.917 | 0.91(2.91) | 917 | 0.91(2.91) | 0.026 |
| Without IV                           | 855(86.63%)       | 56(72.73%)       | 0.917 | 0.91(2.91) | 917 | 0.91(2.91) | 0.026 |
| With palmar hyperlinearity           | 221(22.39%)       | 16(20.78%)       | 0.872 | 0.96(0.55–1.67) | 237 | 1.13(2.86) | 0.468 |
| Without palmar hyperlinearity        | 766(77.61%)       | 61(79.22%)       | 0.97(3.02) | 834 | 0.97(3.02) | 0.468 |
| With keratosis pilaris               | 118(11.96%)       | 15(20.00%)       | 0.084 | 1.67(0.93–3.00) | 134 | 1.69(4.12) | 0.034 |
| Without keratosis pilaris            | 869(88.04%)       | 60(30.00%)       | 0.93(2.77) | 937 | 0.93(2.77) | 0.034 |
| With food intolerance                | 379(43.22%)       | 22(30.14%)       | 0.163 | 0.70(0.43–1.14) | 404 | 0.97(2.90) | 0.322 |
| Without food intolerance             | 498(56.78%)       | 51(30.00%)       | 1.18(3.31) | 552 | 1.18(3.31) | 0.322 |
| With white dermatographism           | 119(14.39%)       | 10(15.87%)       | 0.718 | 1.14(0.57–2.28) | 131 | 1.38(3.43) | 0.109 |
| Without white dermatographism        | 708(85.61%)       | 53(84.13%)       | 0.93(2.94) | 763 | 0.93(2.94) | 0.109 |

P* value: calculated by χ² test; P value: calculated by T test; doi:10.1371/journal.pone.0098235.t005

In addition, we found that c.3321delA was associated with wider age of onset distribution, consistent with the Southern China and the study by Lohr et al. [23] assessing FLG mutations among AD patients in Northern China. FLG R501X and 2282del4 FLG mutations predispose individuals to AD with higher penetrance, consistent with the Southern China study [11]. FLG mutations may be a predisposing factor for the development of FLG null mutations as a result of altered gene expression [31]. A damaged epidermal barrier due to the deletion of FLG results in barrier dysfunction characterized by a decrease in barrier function [32]. Studies have indicated that the white dermatographism [24], keratosis pilaris [25], and food intolerance [26] are putative ultraviolet photoprotectors [27]. Filaggrin degradation can release histidine acting as a nucleation site for the release of an antigen that interacts with IgE-sensitized mast cells, causing allergy [28]. Filaggrin degradation products are important for the maintenance of epidermal barrier hydration. NMFs are less abundant in patients carrying FLG mutations [29]. The loss or reduction of NMFs and impaired expression disrupts barrier formation making filaggrin-deficient skin susceptible to increased transepidermal water loss and容易 by environmental allergens, which can manifest as allergic sensitization [29]. Studies have indicated that the loss of filaggrin expression disrupts barrier formation making filaggrin-deficient skin susceptible to increased transepidermal water loss and easy penetration of allergens through the skin of patients [30]. The destruction of normal epidermal barrier function is considered a key event in allergic sensitization [31]. A damaged epidermal barrier allows allergens to penetrate the skin easily and exposes the allergens to antigen-presenting cells, causing allergy and exposure to allergens [32]. The etiopathogenesis of AD and AD-associated skin xerosis. Therefore, our results are the first to suggest that FLG mutations may be associated with AD associated with food intolerance, consistent with the Southern China study. We also were the first to report that c.3321delA was associated with a large sample of AD patients in Northern China, whereas a similar study using a smaller sample from Northern China did not observe the association. But the German study has reported a strong association [2]. We also were the first to report that c.3321delA was associated with AD consistent with a large sample of AD patients in Northern China, whereas a similar study using a smaller sample from Northern China did not observe the association. But the German study has reported a strong association [2].
epithelia, such as the oral and nasal mucosa, and is also presumed to contribute to the oral epithelial barrier function. Since, allergen through oral can also cause allergy. Our results confirm that skin barrier defects due to FLG mutations play a crucial role in the pathogenesis of other allergic disorders, such as food sensitization. The relationship among AD, asthma, AR and FLG mutations is complex. Common and varying genetic factors for AD and asthma have been reported in the Chinese Han population [32]. A large European cohort study demonstrated that FLG null mutations predispose to allergic phenotypes, such as asthma and AR involved in the atopic march only in the presence of eczema [33]. Several studies have shown that FLG mutations predispose to asthma but only in the context of prior eczema and AD and their families, which indicating that FLG mutations did not have an independent effect on asthma [34–36]. A study [17] in Northern China reported that c.3321delA was associated with AD-associated AR or asthma in stratified analysis \((P = 0.035)\). However, a Polish study also found evidence for the association between combined FLG variants with asthma and AD, and an association between FLG-null variants and atopic asthma was also observed in individuals without AD or a history thereof [37]. In one study in Northern China, no association was observed between the combined FLG mutations and AD-associated asthma or AR; however, an association between the mutation K4671X and AD-associated AR was found [2]. An additional study in Southern China did not find an association between the combined FLG mutations and AD-associated asthma or AR. However, c.3321delA was found more frequently in AD patients without asthma than patients with asthma. In addition, a significant association between c.3321delA and AD patients with asthma was observed \((P = 0.016)\) [11], implying that c.3321delA may serve as a protective factor for asthma that occurs in the context of AD. In this larger sample study, we observed that the c.3321delA allele frequencies in AD patients without asthma or AR were slightly increased compared with AD patients with asthma or AR, but the findings were not statistically significant \((P = 0.86, \text{OR} = 0.96, 95\%CI = 0.64–1.45\) and \(P = 0.297, \text{OR} = 0.82, 95\%CI = 0.56–1.19\), respectively) (Table 3). The negative association between FLG mutations and atopies found in our study may be attributed to sample bias, environmental factors and ethnic differences. The majority of our patients displayed an age of onset for asthma and AR well below the median age at onset. However, we confirmed the previous hypothesis that c.3321delA might be a protective factor for asthma. Additional studies are needed to clarify the relationship between FLG mutations and AD-associated respiratory allergic disorders.

We also performed an analysis regarding orbital darkening, but no significant difference was found, consistent with the Northern China study [2]. AD patients tend to have increased IgE levels. A study from Japan reported that c.3321delA is associated with elevated IgE levels [30], but we were unable to replicate that association in this study, which is consistent with the south China [11] and Polish [37] studies. Above all, according to our results, mutation c.3321delA was associated with several AD phenotypes, and AD patients with c.3321delA tended to have earlier age of onset, though the association is not significant. Since this, we performed further stratified analysis to explore the possible relationship between age of onset and these six AD phenotypes associated with c.3321delA. And we found that AD patients with late age of onset were more likely to accompany with IV, and AD patients with IV or keratosis pilaris tended to have later age of onset in AD. This may be due to the later predilection ages of IV and keratosis pilaris than that of AD.

Of course, there are some limitations in our study. Our study is limited by all cases and controls being of Chinese Han and it is possible that the FLG mutations may not exert the same effect in other races of China and other Asian countries. Our sample size was not large enough, so that most (92.59%) of our patients were early age of onset (≤2 years), and only 13 patients were homozygous, which may lead to minor bias. In addition, the phenotypes-genotypes association analysis in current study was just focused on AD patients, and all the results about phenotypes-genotypes relationship were in the presence of AD. In future study, we will perform the associated analyses of c.3321delA in the non-AD group, such as groups of IV, asthma and AR, et al.

In conclusion, our study confirmed that the FLG mutation c.3321delA was associated with AD under an additive genetic model in a large Chinese cohort. In addition, we observed a correlation between c.3321delA and various clinical features of AD, and we demonstrated that c.3321delA has an effect on these phenotypes in the context of AD. These findings may help to further define the role of FLG in AD susceptibility, thereby assisting in the categorization of various subtypes of the disease and building the foundation for genetic diagnosis and personalized treatment for patients with AD in the near future.

Acknowledgments

We thank all study participants and all the volunteers who have so willingly participated in this study, thus make this study possible.

Author Contributions

Conceived and designed the experiments: LS X. Zhang SY. Performed the experiments: LM LW XJ JZ JG BL XF YC WY WZ BW DD CS HC. Analyzed the data: X. Zuo HT. Wrote the paper: LM. Rectified the manuscript: LM HT LS XT.

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