Prevalence of FLG loss-of-function mutations R501X, 2282del4, and R2447X in Spanish children with atopic dermatitis.

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Prevalence of *FLG* loss-of-function mutations R501X, 2282del4, and R2447X in Spanish children with atopic dermatitis

Abstract

Background/Objectives: Atopic dermatitis (AD) is the most prevalent inflammatory skin disorder, and is often associated with a personal or family history of atopic disease. The presence of loss-of-function mutations in the filaggrin gene (*FLG*) is the main predisposing factor for AD. *FLG* mutations show ethnic and geographical variations, even between European populations. We sought to determine the frequency of the 3 most common *FLG* null mutations in a population of Spanish children consisting of healthy controls and AD patients. We also investigated the association between these 3 *FLG* mutations and AD.
Methods: A total of 214 participants (111 AD patients and 103 healthy controls) were enrolled in this study. Genotyping for 3 *FLG* null mutations (R501X, 2282del4, and R2447X) was performed by conventional Sanger sequencing.
Results: The combined mutation frequency was 1.9% in the control group and 12.6% in the AD group. The most common *FLG* mutation in AD patients was R501X (9.9%), followed by R2447X (2.7%) and 2282del4 (1.8%).
Conclusion: These findings further our understanding of the prevalence of *FLG* null mutations in the Spanish population, and suggest that the frequency of *FLG* mutations in AD patients in Spain is slightly higher than that of other Mediterranean countries.

Introduction

Atopic dermatitis (AD) is the most prevalent inflammatory skin disorder, and is often associated with a personal or family history of atopic disease, allergic rhinitis, and asthma. Although the pathophysiology of AD remains to be fully elucidated, experts agree that a complex relationship between a hyperactive immune system and skin barrier dysfunction, together with environmental factors, may underlie the development of AD (1).

Filaggrin is an epidermal protein that plays crucial roles in forming and maintaining the integrity of the skin barrier. It contributes to the formation of corneocytes, and its intracellular metabolites (urocanic acid and pyrrolidone-5-carboxylic acid) are key components of the skin's natural moisturizing factor and are critical for hydration, pH maintenance, and UV-B protection (2). Loss-of-function mutations in the filaggrin gene (*FLG*, OMIM 135940) result in reduced or absent levels of filaggrin protein and its degradation products (3), and are the primary predisposing factor for AD (4), underscoring the role of skin barrier disorders in the pathogenesis of AD.

FLG mutations show ethnic and geographical variations. The global prevalence of *FLG* mutations is greater in European versus Asian populations (7.7% vs 3%) (5, 6), and

varies considerably among European (2) and Asian (7) populations of AD patients. In the United States, the prevalence of FLG mutations varies among the different ethnic groups. FLG mutations are 6 times less common in African American than in European American patients (5.8% vs 27.5%), even in patients with severe AD (8, 9).

The 3 most frequent FLG null mutations reported in studies of European populations are R501X, 2282del4, and R2447X, with prevalences ranging from 7% to 10% (2, 10). These mutations are significantly associated with AD (10). There are marked differences in the prevalence of *FLG* mutations detected in northern versus southern European populations: in certain northern European populations of AD patients the prevalence of *FLG* mutations ranges from 25–50% (4, 5), while in southern European populations *FLG* mutations are uncommon or even absent, with rates ranging from 0.5% to 4% (11-14).

Only 1 study has assessed the prevalence of *FLG* mutations in a Spanish population, specifically in a cohort of asthma patients and a corresponding control group (15). That study revealed frequencies of 2% and 1% for R501X and 2282del4, respectively, in the control group, and lower frequencies in the asthma cohort (1% and absent, respectively). In this study, we sought to characterize the prevalence of these null mutations in a group of children diagnosed with AD and in a group of healthy controls.

Material and methods

Study population

We conducted a case-control pilot study of patients aged between 2 months and 14 years who had current AD at the time of inclusion, skin phototype 2 to 4, Mediterranean phenotype, and both parents from Spanish origin. Participants were recruited between January 2011 and December 2012 in the Departments of Dermatology and Pediatric Allergy of the Hospital San Jorge (Huesca, Spain), primary care centers in Huesca city, and the Department of Dermatology of the Hospital Niño Jesús (Madrid, Spain). Control subjects were recruited at the University Hospital Miguel Servet (Zaragoza, Spain), applying the following exclusion criteria: family history of atopy or allergic diseases, symptoms of atopic eczema, asthma or hay fever, food or pollen allergies or other environmental allergens (e.g. animals).

AD severity was scored using the scoring atopic dermatitis (SCORAD) index (16, 17), and patients classified as mild (SCORAD <15), moderate (SCORAD 15-40), or severe (SCORAD >40) (11, 18). Personal history of atopic diseases, including asthma and allergic rhinitis, was also recorded.

The study protocol was approved by the Aragon Ethical Committee for Clinical Research (PI08/81). Written informed consent was obtained from all participants, or their guardians, before inclusion.

Genomic studies

Genomic DNA was obtained from peripheral blood leukocytes extracted from EDTA whole blood samples using the QIAamp DNA Blood Mini Kit (QIAGEN, Germany) and an automated EZ1 biorobot (QIAGEN, Germany). Genotyping for the *FLG* mutations R501X, 2282del4, and R2447X was performed by PCR fragment amplification followed by conventional Sanger sequencing. The primers used were designed based on the findings of previous studies and modified to our PCR optimization protocol (11).

Data analyses

Descriptive statistics for quantitative values were expressed as the mean and standard deviation (SD), in accordance with the data distribution. Frequencies and percentages were used to describe categorical variables. Chi-squared or Fisher's exact tests were used to assess associations between *FLG* mutations and AD, as well as AD-associated variables, including SCORAD index and associated atopic diseases. The association of genotypes or alleles with AD was assessed by calculating Odds Ratio (OR) and 95% confidence intervals. The level of statistical significance was set at $p<0.05$. Statistical analyses were performed using SPSS version 19 (SPSS Inc., Chicago, IL, USA).

Results

Clinical features

Table 1 summarizes the clinical characteristics of the sample. The total number of participants enrolled was 214; 111 with a diagnosis of AD and 103 healthy controls (mean [SD] age: 5.51 [3.93] and 9.72 [6.22] years, respectively). Based on SCORAD index, 20 patients (18%) had mild AD, 70 (63%) had moderate AD, and 21 (19%) had severe AD. In the AD group, the frequency of asthma and rhinitis was 30% and 17%, respectively. Both conditions were absent in the control group.

The frequency and distribution of *FLG* mutations R501X, 2282del14, and R2447X and of the combined *FLG* genotype for each group are shown in Table 2. All 3 mutations were detected in the AD group, whereas only R501X and 2282del4 were identified in 2 controls. *FLG* mutations were detected in 14 AD patients, all of whom were het-

erozygous for 1 of the null mutations, and 2 of whom were compound heterozygous carriers of 2 different *FLG* mutations (see Supplementary Material). Mutations R501X, 2282del4, and R2447X were carried by 11 (9.9%), 2 (1.8%), and 3 (2.7%) AD patients, respectively. The combined frequency of *FLG* mutations was 1.9% for the control population and 12.6% in AD patients.

Analysis of the combination of all 3 *FLG* mutations of interest revealed a significant association with susceptibility to AD (p=0.006). A significant association with AD was observed only for the R501X mutation (OR= 11.220, p=0.005) (Table 2).

Although most of the *FLG* mutations were detected in patients with moderate AD (n=11), we found no significant association between AD severity and *FLG* mutations, either individually or combined (Table 3), and no association between *FLG* null mutations and other atopic diseases such as asthma or rhinitis (Table 3).

Discussion

This is the first study to evaluate the frequency of the most common *FLG* loss-of-function mutations (R501X, 2282del4, and R2447X) in a population of Spanish AD patients. The combined frequency revealed a prevalence of 1.9% in the control group and 12.6% in AD patients. R501X (9.9%) was the most common *FLG* mutation in our AD cohort, followed by R2447X (2.7%) and 2282del4 (1.8%). These results are similar to those reported by Cubero et al. in a population of asthmatic patients from the same geographical area (14). Those authors reported frequencies of 2% and 1% for R501X and 2282del4, respectively, and, in line with our findings, no association between these main *FLG* null-mutations and asthma.

The frequency of *FLG* loss-of-function mutations in our healthy control population (1.9%) is similar to that reported in previous studies of Mediterranean or southern European populations, including Italian (0.6%) (13), Croatian (2.6%) (14), Spanish (3.0%) (15), and French (4%) (11) cohorts. However, it is lower than that reported in northern European countries, including Germany (7.4%) (19) and England (8.8%) (20) (Table 4).

In our AD cohort, the prevalence of the *FLG* loss-of-function mutations R501X, 2282del4, and R2447X (9.9%, 1.8%, and 2.7%, respectively) was significantly lower than that reported for the same 3 mutations in AD patients in northern European countries, including England (42.3%) (20) and Germany (22.9%) (19, 21). However, frequencies similar to those reported here have been reported in AD cohorts in Poland (10.3%) (18) and in other Germany studies (11.0%) (22) which reflects the complexity of the distribution. The only two published studies of Mediterranean AD cohort

(French and Italian) reported mutation frequencies similar to (in case of French cohort) or slightly lower (in case of Italian cohort), than those reported here (11, 12) (Table 4). Our results are not comparable to those reported in the North American population, since there are considerable ethnic differences.

Our findings confirm the association between *FLG* mutations and AD susceptibility: a child carrying one of these 3 mutations is more likely to develop AD than one without. These results are in agreement with previous reports.

A limitation of our study is the small sample size. A key strength of the study is that it is the first such study of AD patients in Spain. One explanation for the low frequency of *FLG* mutations in our cohort is that decreased FLG expression caused by either genetic mutations or skin inflammation can induce filaggrin deficiency. This is one potential reason why it has not been possible in this study to establish an association with other allergic diseases.

In conclusion, our study furthers our understanding of the prevalence of *FLG* mutations in Spanish AD patients, and shows that the frequency of the most common *FLG* null mutations in this cohort is slightly higher than that previously reported in other Mediterranean populations. Furthermore, our findings corroborate the previously reported association between these *FLG* mutations and AD.

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