Allergol Immunopathol (Madr). 2016;44(4):341–345

Allergologia et immunopathologia

Sociedad Española de Inmunología Clínica, Alergología y Asma Pediátrica

www.elsevier.es/ai

ORIGINAL ARTICLE

FLG single nucleotide polymorphisms in chronic idiopathic urticaria

S. Soltani[a], A. Saghazadeh[a], M. Movahedi[b], M. Tavakol[c], M. Sadr[a], E. Farhadi[d], N. Rezaei[a,e,f,*]

[a] *Molecular Immunology Research Center, School of Medicine,Tehran University of Medical Sciences, Tehran, Iran*
[b] *Pediatrics Center of Excellence, Children's Medical Center, Tehran University of Medical Sciences, Tehran, Iran*
[c] *Department of Allergy and Clinical Immunology, Shahid Bahonar Hospital, Alborz University of Medical Sciences, Karaj, Iran*
[d] *Hematology Department, School of Allied Medical Science, Iran University of Medical Sciences, Tehran, Iran*
[e] *Research Center for Immunodeficiencies, Children's Medical Center, Tehran University of Medical Sciences, Tehran, Iran*
[f] *Universal Scientific Education and Research Network (USERN), Tehran, Iran*

Received 9 July 2015; accepted 30 September 2015
Available online 13 January 2016

KEYWORDS
FLG;
Filaggrin;
SNP;
Single nucleotide polymorphism;
Chronic idiopathic urticaria

Abstract
Background: Filaggrin (FLG), which is formed from profilaggrin protein during epidermal terminal differentiation, is a prerequisite to squame biogenesis and thus for perfect formation of the skin barrier. Yet, the relationship between genetic polymorphisms of FLG and chronic idiopathic urticaria (CIU) has not been investigated.
Methods: The study population consisted of 93 CIU patients and 93 healthy control subjects without a history of allergic, autoimmune or any other systemic disease. Five single nucleotide polymorphisms (SNPs) of FLG were investigated: rs2485518, rs3126065, rs2786680, rs3814300, and rs3814299.
Results: For all the investigated polymorphisms, 100% of both CIU patients and control subjects exhibited one given allele and consequently one given genotype as following: A/A genotype for two SNPs, rs3126065 and rs2786680, C/C genotype for two SNPs, rs2485518 and rs3814300, and G/G genotype for one SNP rs3814299 of FLG, and hence no association was found between either allele frequencies or genotype distributions of FLG SNPs and CIU in an Iranian population.
Conclusions: The present study examined the possible relationship between SNPs of FLG and CIU for the first time, and demonstrated that none of five investigated SNPs (rs2485518, rs3126065, rs2786680, rs3814300, and rs3814299) are correlated with CIU in an Iranian population. Further investigations are required to address whether ethnicity/race impacts on relationship between SNPs of FLG and CIU.

* Corresponding author.
E-mail address: rezaei_nima@tums.ac.ir (N. Rezaei).

http://dx.doi.org/10.1016/j.aller.2015.09.002

Introduction

Chronic urticaria (CU) is characterised by widespread smooth, oedematous wheals occurring daily or almost daily for more than six weeks.[10] Angio-oedema seems a common occurrence in CU, due to its observation in more than one third of patients.[17] In approximately 20% of CU adults, the disease duration is longer than one year, and women are more likely to develop CU than men.[8] It is not altogether surprising that the heavy burden of CU falls on many aspects of daily life, including mobility, sleep and social interactions.[26] CU is categorised under three main headings, physical urticaria, urticarial vasculitis and chronic idiopathic urticaria (CIU). Detection of histamine-releasing IgG autoantibodies against either the high-affinity IgE receptor FcεR1 or IgE in sera of CIU patients has led to label 25–50% of CIU patients as autoimmune urticaria and thereby to manage more effectively the disease in these patients by means of problem solving-based strategies such as plasmapheresis.[9,11,14,25] Additionally, complement system, cytokines, chemokines and adhesion molecules are of pathogenic findings in CU, which collectively cause in recruiting a wide range of immune cells, e.g. CD^{4+} lymphocytes, neutrophils, basophils and monocytes.[17] In addition to the immunological basis of CIU, several studies have suggested that the generation of oxidative stress damage, as assessed by levels of reactive oxygen species and activity of superoxide dismutase, may be a key stimulus to the pathogenesis of CIU.[5] More interestingly, it attracts a great deal of attention that most CIU patients have determined stress as the exact cause of their disease.[29]

Nevertheless, despite nearly a century of research, the chief factor(s) of CU remain unknown; there is, thus, a conspicuous absence of efficient treatments for a considerable number of cases, who are still considered idiopathic. Findings of substantially increased risk of CIU in people with at least one positive first-degree history of CIU than in general population,[1] in addition to the proven genetic associations provided by population-based genetic association studies,[27,37] do really appreciate the value of genetic investigations to unveil the nature of CIU.

The protein filaggrin (FLG) acts as an intermediate filament-associated protein and aggregates keratin intermediate filaments in mammalian epidermis; FLG is, thus, required to complete correctly keratinocyte construction and to properly maintain the permeability of the epidermal barrier.[12] The gene encoding FLG located on chromosome 1q21.3 is the most associated gene with atopic dermatitis (AD) (for review see Ref. [2]). Regarding CIU, a recent finding has indicated significant upregulation of the FLG expression in lesional CIU patients more than in AD patients, and as well significant increased staining intensity of FLG in lesional CIU patients compared with either AD patients or control subjects.[41] Furthermore, the research demonstrated a significant association between filaggrin staining intensity and urticaria activity score.[41] However, the relationship between genetic polymorphisms of FLG and CIU has yet to be investigated. We accomplished the present case-control study to address the question as to whether single nucleotide polymorphisms (SNPs) of FLG are associated with susceptibility to CIU.

Materials and methods

Subjects

Blood samples were obtained from 93 Iranian CIU patients who were referred to the Children's Medical Centre, the Paediatrics Centre of Excellence in Tehran, Iran. The group of control subjects was set up from healthy members without a history of allergic, autoimmune or any other systemic disease. All CIU cases were diagnosed according to the standard international criteria,[20] i.e. the wheals lasting for six weeks or longer with occurrences of at least two times a week and its underlying cause remained unclear despite the appropriate investigations. Patients with physical urticaria, food/medication induced allergy or urticarial vasculitis were excluded. Other exclusion criteria were urticaria lesions due to infections, environmental agents, stress or cholinergic stimulation and heat/cold induced urticaria, dermatographic urticaria and pressure induced urticaria. To correctly exclude the aforementioned aetiologies, the onset and duration of lesions, characteristics of lesions, distribution of lesions, and history of any associated disease or allergies, drug history and complement deficiencies were considered. Specific laboratory tests were performed to approve the suspicious diagnosis, for instance C3, C4, CH50 and C1 inhibitor (C1-INH) for angio-oedema. DNA was extracted from nucleated cells according to the phenol–chloroform protocol.[7]

All individuals enrolled in the study gave their written consent. This study was approved by the ethics committee of Tehran University of Medical Sciences (TUMS).

Genotyping

In the present study, FLG polymorphisms were identified by means of polymerase chain reaction with the sequence specific primers (PCR-SSP) assay (PCR-SSP kit, Heidelberg University, Heidelberg, Germany). A PCR Techne Flexigene apparatus (Rosche, Cambridge, UK) was applied for the amplification of extracted DNA. PCR products were observed by 2% agarose gelelectrophoresis and a picture was taken after visualisation with a UV transilluminator. Five SNPs of FLG were investigated: rs2485518, rs3126065, rs2786680, rs3814300, and rs3814299. Laboratory personnel were blinded to the study.

Statistical analysis

For each one of the five investigated SNPs, genotype distributions and allele frequencies were calculated by direct gene counting. Pearson's chi-squared test was used in examining the differences in the distribution between CIU cases and healthy controls. All the statistical analyses of the present study were implemented using the Epi Info statistical software (version 6.2, World Health Organisation, Geneva, Switzerland).

Results

Allele and genotype frequencies

The allele frequencies and genotype distributions of FLG polymorphisms for CIU cases and control subjects are