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Research paper

The study of filaggrin gene mutations and copy number variation in atopic dermatitis patients from Volga-Ural region of Russia

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ABSTRACT

Atopic dermatitis (AD) is a chronic inflammatory skin disease characterized by age-specific localization, dryness, itch and hypersensitivity to allergens. In our study, we investigated *FLG* gene mutations and CNVs in AD patients and control subjects of different ethnic origin from Volga-Ural region. AD group included 303 patients (177 Russians, 126 Tatars). Control group consisted of 261 healthy individuals (152 Russians, 109 Tatars). The study revealed 66 *FLG* mutation carriers and demonstrated an association between *c.2282del4* deletion and AD development in Russians and Tatars of Volga-Ural region of Russia. In the analysis of the FLG gene CNVs, the most common was 10-repeat allele in both Russian and Tatar patients and controls. We were unable to find any significant difference in CNV repeats count between AD patients and control individuals.

1. Introduction

Atopic dermatitis (AD) is a chronic inflammatory skin disease characterized by age-specific localization, dryness, itch and hypersensitivity to allergens. The AD incidence has grown significantly during the last decades and has reached 10–20% on average. AD generally prevails in children and usually arises before age one but can also continue into adulthood. AD is mostly the first manifestation of an allergy and up to 50% of children with AD develop asthma or allergic rhinitis later.

AD is a complex disease evolving from the interaction of genetic predisposition and atopy with different environmental stimuli of which allergens have the most important influence.

The mechanisms underlying the pathogenesis of AD are still not clear but to date allergic immune response and epidermal barrier defects are considered the main of them. Molecular genetic investigations showed the significant role of the great number of genes in AD development including genes coding for cytokines participating in the atopic reactions (*IL4, IL10, IL13, IL18, IL25, IL33, TSLP*), epidermal proteins (*FLG, SPINK5*), pattern-recognition receptors (*TLR*) and others (Chavanas et al., 2000; Tsunemi et al., 2002; Ahmad-Nejad et al., 2004; Hosomi et al., 2004; Palmer et al., 2006; Sohn et al., 2007; Esparza-Gordillo et al., 2009; Paternoster et al., 2015).

In recent years, epidermal barrier disruption is considered to play the main role in AD development. Barrier function abnormality leads to skin permeability towards allergens. Intensive allergen penetration results in a sensitization of an organism and causes the allergic response. Besides, skin barrier defects cause excessive transepidermal water loss and thus develops water balance disturbance. Water is crucial in tissues flexibility and integrity and in enzymatic processes. Essential water level is maintained by natural moisturizing factor (NMF) consisting from low molecular weight compounds. The latter are produced for the great part from filaggrin. NMF levels are known to be reduced in dry skin (Horii et al., 1989). There is also an assumption that FLG breakdown products prevent increased susceptibility to UV (Harding et al., 2013).

Filaggrin is the main component of keratohyalin granules of the stratum corneum and is critical in the terminal differentiation of epidermis. Together with keratin filaments and other proteins, it forms cornified cell envelope, which provides full skin barrier function. Moreover, it has been reported that filaggrin loss-of-function mutations may cause skin pH change (Jungersted et al., 2010; Kezic et al., 2012) though data are inconsistent (Bandier et al., 2014). An alteration of skin pH may be due to decrease in levels of the major filaggrin breakdown products urocanic and 2-pyrrolidone-5-carboxylic acids that cause alkalinization of the skin pH.

Filaggrin gene (*FLG*) is located on chromosome 1q21 in the epidermal differentiation complex genes cluster. *FLG* gene contains three exons.

Abbreviations: AD, atopic dermatitis; CNV, copy number variations; FLG, filaggrin; NMF, natural moisturizing factor; TLR, toll-like receptor; RFLP, restriction fragment length polymorphism; PCR, polymerase chain reaction; OR, odds ratio; 95% CI, confidence interval.

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The first exon codes for 5′-untranslated region, the second one includes initiation codon and the third exon comprises highly homologous (almost 100% at DNA level) repeat units. The number of filaggrin units is genetically determined, and different individuals have 10, 11 or 12 repeat alleles (copy number variation, CNV) depending on duplications of 8th and/or 10th units. The number of the repeats defines synthesized protein quantity so that it is involved in the AD development. It has been shown that AD patients have lower filaggrin repeats number compared to healthy individuals (Brown et al., 2012).

Filaggrin gene mutations are the main known genetic risk factors of AD and other allergic diseases concomitantly with AD. Loss-of-function mutations in the *FLG* occur in up to 50% of patients with moderate to severe AD and in up to 15% of patients with mild AD (Brown et al., 2009; Rodriguez et al., 2009). There are >77 *FLG* gene mutations known today of which 27 are present in AD cases and the spectrum of mutations differs in different ethnic groups (www.hgmd.org). The vast majority of them are located in the third exon of the *FLG* gene and lead to early stop of protein translation. As a result, defective protein forms so that stratum corneum becomes impaired and skin barrier function damaged.

The most prevalent mutations in Northern Europeans are *c.2282del4, p.R501X, p.R2447X, c.3702delG, p.Ser3247X* (Sandilands et al., 2007), in Asians — *c.441delA*, *c.1249insG*, *c.3321delA*, *p.Ser2554X c.7945delA, p.Gln2147X, p.Glu2422X* (Akiyama, 2010; Ma et al., 2010; Li et al., 2013).

To date, marked variations among populations are shown in susceptibility to hereditary and multifactorial diseases, including allergy, diversity in genetic polymorphisms is considerable as well. This was also revealed during genetic analyses on different ethnic groups from different regions of Russia.

According to previous investigations of our colleagues, in the ethnic groups of Volga-Ural region of Russia mutations specific for European population are usually found (Dzhemileva et al., 2010; Khidiyatova et al., 2012; Prokofyeva et al., 2012). Population genetic studies showed that Russians and Tatars have mostly West Eurasian genetic component (cluster) in their structure, and Asian component is insignificant (Yunusbayev et al., 2015). Studies on mitochondrial DNA and Y-chromosome also revealed low frequencies of haplogroups specific for Asian populations in ethnic groups we studied (Bermisheva et al., 2002; Trofimova et al., 2015). Taking this into consideration we aimed to explore the frequency of *c.2282del4, p.R501X* and *p.R2447X* mutations and CNVs of the *FLG* gene in AD patients and control subjects of Russian and Tatar ethnic origin living in Volga-Ural region of Russia.

2. Materials and methods

AD group included 303 patients (177 Russians, 126 Tatars) aged 1 to 50 (mean age 13.29 yr) from Republican Dermatovenereological Dispensary and Bashkir State Medical University Clinic. AD was diagnosed by qualified physicians on the basis of clinical, laboratory and supplementary findings, according to criteria proposed by Hanifin and Rajka (1980).

Severity of AD was assessed using the SCORing Atopic Dermatitis index.

(SCORAD index) and categorized into mild (<25), moderate (25–50) or severe (>50) AD (Stalder and Taieb, 1993). Then patients were divided into two groups: 1) mild AD and moderate AD (153 Russians, 106 Tatars); 2) severe AD (24 Russians, 20 Tatars). Among patients, 149 have only AD symptoms (82 Russians, 67 Tatars) and 154 have also concomitant allergic diseases like asthma, allergic rhinitis or allergic conjunctivitis (95 Russians, 59 Tatars).

Control group consisted of age, gender and ethnicity matched healthy subjects (152 Russians, 109 Tatars) without past or present history of atopic manifestations. Individuals' ethnic background was determined by ascertaining the ethnicity of their grandparents.

All the participants gave written informed consent. The Research Ethics Committee of the Institute of Biochemistry and Genetics of Ufa Scientific Center of Russian Academy of Sciences approved the study.

2.1. Genotyping of mutations and CNV detection in the FLG gene

Genomic DNA was isolated from peripheral blood using standard phenol-chloroform extraction. Genotyping of the *FLG* mutations was carried out by a PCR and restriction enzyme digestion (RFLP) method (Smith et al., 2006; Sandilands et al., 2007). Detection of CNV was performed by PCR followed by gel electrophoresis (Sandilands et al., 2007). Statistical analyses were performed using Excel (Microsoft).

3. Results

We conducted the analysis of *c.2282del4*, *p.Arg501X* and *p.Arg2447X* mutations of the *FLG* gene in AD patients and control individuals living in the Volga-Ural region of Russia. In total, 66 of investigated AD patients (15.49%) have *FLG* gene mutations.

3.1. Analysis of the FLG mutations in Russians

Russians have significant difference in frequencies of *c.2282del4* mutation between patients and control individuals (Table 1). Allelic frequency of the deletion was 6.03% in patients and 0.67% in controls ($p = 0.00023$, OR = 9.57 (CI 95% 2.22–41.15)).

Mutation frequency in patients with AD of different severity also varied. In group of Russians with moderate AD allelic frequency of *c.2282del4* was 5.96%, among Russians with severe AD the allelic frequency of the mutation was 6.25%.

As mentioned before, patients included individuals with only AD symptoms and individuals having AD and concomitant allergic diseases. The allelic frequency of the deletion was 6.79% in Russians having only AD and 5.32% — in group of Russian AD patients with concomitant allergic diseases.

The second investigated mutation, *p.R501X*, was rather rare in the analyzed groups. In Russians, heterozygote mutation was revealed in 3 out of 177 AD patients (1.69% of individuals), all have moderate AD and two of them have concomitant allergic diseases. In control group, we found four heterozygous mutation carriers out of 152 individuals (2.63% of subjects). The allelic frequency of *p.R501X* was 0.90% in patients and 1.36% in controls.

We failed to find any *p.R2447X* mutation carrier among Russian AD patients.

3.2. Analysis of the FLG mutations in Tatars

Significant differences in *c.2282del4* frequency were revealed between Tatar AD patients and control individuals (Table 1). Allele frequency of *c.2282del4* was 9.35% in patients and 0.46% in controls ($p = 0.000016$, OR = 22.38 (CI 95% 3.00–167.16)).

In Tatars with moderate AD the deletion appeared at allelic frequency of 8.74%. The allelic frequency of the mutation in the sample of individuals with severe AD was equal to 12.50%.

In patients of Tatar ethnic origin without concomitant allergic diseases *c.2282del4* was detected with the allelic frequency of 10.45%. The allelic frequency of the deletion among Tatar AD patients with concomitant allergic diseases was 8.04%.

Another *FLG* gene mutation, *p.R501X*, was found in 2 out of 117 Tatar AD patients (1.71% of individuals), both having moderate AD and concomitant allergic diseases. There was one heterozygote for this mutation in control group (0.94% of persons). The allelic frequency of the mutation in AD patients was 0.85% and in controls — 0.47%.

The *p.R2447X* mutation was found in 4 Tatar AD patients out of 114 (3.51%) and in two respective controls out of 75 (2.67%). The allelic frequency of *p.R2447X* was 1.75% in patients, and 1.33% in controls groups.