

## Low frequency of filaggrin null mutations in Croatia and their relation with allergic diseases

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### Summary

Filaggrin gene (*FLG*) null mutations are considered associated with atopic dermatitis. This study was conducted to determine the prevalence of *FLG* null mutations R501X, 2282del4, R2447X and S3247X in the Croatian population and their role in the occurrence of allergic diseases including atopic dermatitis, allergic rhinitis, asthma and allergic contact dermatitis (ACD). Study enrolled 440 freshmen with defined allergic diseases by means of both present symptoms in International Study of Asthma and Allergies in Childhood questionnaire (relevant respiratory and/or skin symptoms) and markers of allergic sensitization (positive skin prick and/or patch test). *FLG* null mutations were successfully genotyped in 423 students of which 11 (2.6%) were carriers of *FLG* null mutation: 1/423 (0.2%) was heterozygous for R501X and 10/423 (2.4%) were heterozygous for 2282del4. No carriers of R2447X and S3247X mutations were identified. In wild-type *FLG* carriers (412 subjects), atopic dermatitis was present in 45 (11%), allergic rhinitis in 70 (17%) and allergic asthma in 29 (7%) students. Twenty-five of 393 (7%) patch-tested wild-type *FLG* carriers had ACD. Among 11 *FLG* null mutation carriers, four had one or more allergic diseases, and five had reported skin symptoms without defined allergic sensitization (positive skin prick test and/or patch test). *FLG* null mutations were not confirmed as a predictor of analysed allergic diseases, but were confirmed as an independent predictor of skin symptoms (OR 17.19, 95% CI 3.41–86.6,  $P < 0.001$ ). Our results in

general indicate a low frequency of *FLG* null mutations in the studied Croatian population supporting a theory of a latitude-dependent distribution of *FGL* null mutations in Europe, with a decreasing north–south gradient of R501X and 2282del4 mutation frequency. The relation between *FLG* null mutations and skin disorders was confirmed.

### Introduction

Null mutations of the gene encoding filaggrin (*FLG*), located in chromosomal epidermal differentiation complex 1q21, have been considered associated with the development of allergic disorders, predominantly atopic dermatitis (Palmer *et al.*, 2006). Additionally, there is evidence that *FLG* null mutations increase the risk of developing allergic sensitization, allergic rhinitis and asthma associated with atopic dermatitis (van den Oord & Sheikh, 2009).

Filaggrin (filament-aggregating protein) plays a key role in epidermal barrier function by aggregating keratin filaments in *stratum corneum* and upholding *stratum corneum* moisturization. Additionally, breakdown products of filaggrin, urocanic acid and pyrrolidone carboxylic acid are essential in preventing skin dehydration as major components of natural moisturizing factor (Sandilands *et al.*, 2009). Therefore, filaggrin regulates skin permeability for water and small particles such as allergens. Filaggrin defects are implicated in the skin barrier disorders, and experimental research showed that dysfunction of skin barrier could enhance sensitization to allergens and lead to systematic allergic responses such as increased IgE levels and airway hyperreactivity (Spergel *et al.*, 1998). It has been speculated that *FLG* null mutations could increase the risk of contact allergy by allowing greater skin penetration of chemical haptens (Novak *et al.*, 2008).

Filaggrin null mutations are present in up to 10% of Caucasian population in Western Europe and North America (van den Oord & Sheikh, 2009) with the most common null mutations R501X and 2282del4. They represent about 66% of *FLG* mutations and with added R2447X and S3247X cover about 95% of *FLG*

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mutation in the stated population (Palmer *et al.*, 2006; Sandilands *et al.*, 2007).

This study was aimed to present data on prevalence of common *FLG* null mutations R501X, 2282del4, R2447X and S3247X and their relation with allergic diseases in Croatian population.

## Materials and methods

A survey enrolled 440 freshmen at the University of Zagreb (316 women and 124 men, median age 19 years), residents from two Croatian regions, Continental (342 subjects) and Mediterranean (87 subjects). Study had a cross-sectional design and its protocol included modified International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire, skin prick and patch testing and buccal swab collection and analyses. All subjects were recruited on voluntary basis. Each subject was informed about the study protocol and signed a consent form. Study was designed in accordance with Helsinki Declaration and approved by the authorized Ethical Committee.

A modified ISAAC questionnaire (Asher *et al.*, 1995) was completed by the physician for each subject to determine the presence of respiratory and skin allergic symptoms in the previous year and data on family history of allergies. It included questions on symptoms of asthma, rhinitis and dermatitis.

Standard skin prick test (SPT) (Dreborg & Frew, 1993; Bousquet *et al.*, 2012) was performed in all subjects with common inhalatory allergens (Allergopharma, Reinbeck, Germany): grass pollen mixture, birch, hazel and weed (*Ambrosia elatior*, *Artemisia vulgaris*) pollens, house dust mites (HDM) (*Dermatophagoides pteronyssinus*, *D. farinae*), moulds (*Cladosporium herbarum*, *Alternaria alternata*), cat and dog dander and storage mite *Lepidoglyphus destructor*. SPT included testing with positive control solution of histamine (10 mg mL<sup>-1</sup>) and negative buffer control solution (Allergopharma). Skin reaction (weal) was evaluated after 15 min. Mean wheal diameter (D+d)/2 ('D' is the largest longitudinal diameter and 'd' is its midpoint orthogonal diameter) that was larger than negative control by more than 3 mm was considered positive.

Standard patch testing (Wahlberg, 1992) was performed in 394 subjects using European standard series (EECDRG, 1995) of commercial contact allergens (Brial, Greven, Germany) on Curatest (Lohmann, Neuwied, Germany) plaster strips. After a 2-day occlusion, skin reactions were read on 2nd and the 3rd day following application of allergens. Erythema, induration and vesicles ('++' or '+++') presented on the 3rd day were considered signs of positive reaction.

Subjects with allergic diseases were defined through both reported allergic symptoms and positive skin prick or patch testing as follows:

- 1 Atopic dermatitis was defined with both reported skin symptoms and positive skin prick test to at least one common inhalatory allergen.

- 2 Allergic contact dermatitis was defined with both reported skin symptoms and positive patch test to at least one standard contact allergen.

- (a). Subjects with skin symptoms were defined with two or more reported symptoms including redness, itching, vesicles and rash (Johansson *et al.*, 2004).

- 3 Allergic rhinitis was defined with both reported nasal symptoms and positive skin prick test to at least one common inhalatory allergen.

- (a). Subjects with nasal symptoms were defined with two or more reported symptoms including runny nose, itchy nose, blocked nose and sneezing (Johansson *et al.*, 2004; Bousquet *et al.*, 2008).

- 4 Allergic asthma was defined with both reported asthma symptoms and positive skin prick test to at least one common inhalatory allergen.

- (a). Subjects with asthma symptoms were defined with reported wheezing and/or dyspnoea (Johansson *et al.*, 2004; GINA (2011)).

## DNA sampling and isolation

In all subjects, genomic DNA was extracted from buccal swab samples using proteinase K (Invitrogen, Carlsbad, CA, USA) digestion and DNA isolation kit (Puregene<sup>®</sup> Cell Lysis Solution; Gentra Systems, Minneapolis, MN, USA), following the manufacturer's procedures as described previously (de Jongh *et al.*, 2008).

## Genotyping of *FLG* null mutations

Filaggrin mutations R501X and 2282del4 were genotyped using previously reported methods (Palmer *et al.*, 2006; de Jongh *et al.*, 2008). Genotyping for R2447X (sequence: caccacAagcaggca[C/T]gagacagctccaggc) and S3247X (sequence: ctgTtcaggagcagt[A/C]aaggcagcgctccag) was performed by KBioscience (<http://www.kbioscience.co.uk>) using KASP SNP genotyping system, a homogeneous fluorescent resonance energy transfer (FRET)-based system, coupled with competitive allele-specific PCR.

## Statistical analysis

Descriptive statistics was used to assess the characteristics of the subjects, which were carriers of wild-type and *FLG* null mutations. Possible deviation from Hardy-Weinberg equilibrium was tested by Pearson's chi-square test. The frequency of *FLG* null mutations in two Croatian regions was also evaluated by Pearson's chi-square test. Possible association of *FLG* null mutations with the presence of allergic skin diseases (atopic dermatitis and/or allergic contact dermatitis) was evaluated by calculating crude risk ratio and by multiple regression analysis controlled for gender and

family history of skin allergies. The dependent variable was the presence of allergic skin disease versus absence of any skin or respiratory allergic disease. Possible association of *FLG* null mutations with the presence of any type of skin symptoms (both allergic and nonallergic) was evaluated by calculating crude risk ratio and by multiple logistic regression analysis with stated skin symptoms as dependent variable and gender, family history of allergic skin diseases, positive SPT and positive patch test as predictors. Multiple logistic regression analysis with stated respiratory symptoms as dependent variables was performed to evaluate their relationship with *FLG* null mutations controlled for gender, family history of allergic respiratory diseases and positive SPT. Statistical analysis was performed with STATA/SE 10.1 for Windows (Stata Corp LP, College Station, TX, USA).

## Results

In this study, four *FLG* mutations were genotyped in 440 students. Genotyping success rate for all *FLG* null mutations was 96% and these subjects ( $N = 423$ ) underwent statistical analysis. The distribution of *FLG* variants did not deviate from Hardy–Weinberg equilibrium.

There were 412 (97.4%) subjects determined as wild-type *FLG* carriers with respect to 2282del4, R501X, R2447X and S3247X mutations and 11 (2.6%) subjects identified as *FLG* null mutation carriers: 10 (2.4%) were heterozygous for 2282del4 and one (0.2%) was heterozygous for R501X. No carriers of R2447X or S3247X mutations were identified. Homozygous or compound heterozygous carriers for 2282del4 and R501X were not identified. *FLG* null mutations were observed in similar proportion in subjects from both Croatian regions, that is, in 2.4% of subjects from Continental region and in 3.5% of subjects from Mediterranean region (Pearson's chi-square = 0.305, Fisher's exact  $P = 0.704$ ).

In total sample (440 subjects), atopic dermatitis was present in 48 students (11%), allergic rhinitis in 74 students (17%) and allergic asthma in 31 students (7%). Allergic contact dermatitis was present in 26 of 394 patch-tested students (7%). The most prevalent contact allergen was nickel sulphate, which was found positive in 13 of 26 (50%) subjects with allergic contact dermatitis. One or more of these allergic diseases was present in 110 students (28%).

The distribution of allergic diseases within the wild-type *FLG* carriers was similar to their distribution in a total sample (Fig. 1). Characteristics of subjects with determined *FLG* null mutations are listed in Table 1. Among 11 *FLG* null mutation carriers, four had one or more allergic diseases (two had atopic dermatitis and allergic rhinitis and/or asthma, one had only atopic dermatitis, one had only allergic contact dermatitis), and five reported skin symptoms without defined allergic sensitization (positive SPT and/or patch test).

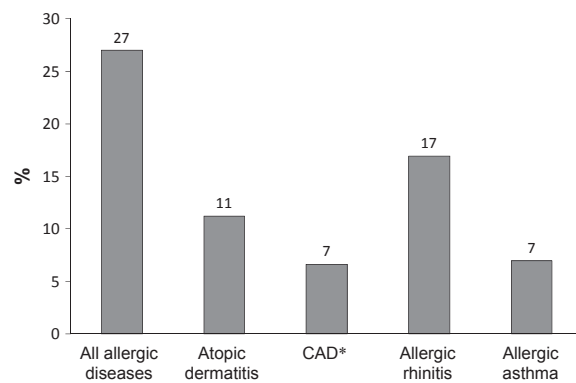


**Figure 1.** The prevalence of allergic diseases in wild-type filaggrin carriers ( $N = 412$ ). CAD = contact allergic dermatitis; \* $N = 393$  patch-tested subjects. Allergic diseases are defined as in Table 1.

No *FLG* null mutation carriers had respiratory allergic disease without concomitant allergic skin disease. The prevalence of allergic skin diseases (atopic dermatitis or allergic contact dermatitis) was higher among *FLG* null mutation carriers (40%, that is, four of ten subjects tested with SPT and patch test) compared to wild-type carriers (15%), with 2.6 times higher crude risk ratio (95% confidence interval 1.2–5.8,  $P = 0.034$ ). The risk was even higher for the presence of any type of skin symptoms (allergic and nonallergic), namely *FLG* null mutation carriers were at four times higher risk compared to wild-type carriers (95% confidence interval 2.9–5.6,  $P < 0.001$ ). The separate risk for any specific allergic skin or respiratory diseases has not been calculated due to small statistical power ( $<0.40$ ).

In the model of multiple logistic regression analysis ( $P_{\text{model}} = 0.004$ , Pseudo  $R^2 = 0.043$ ), the presence of allergic skin disease (atopic dermatitis and/or allergic contact dermatitis) was not associated with *FLG* null mutations ( $P = 0.100$ ) and was positively correlated with family history of skin allergies (OR 3.52, 95% CI 1.52–8.13,  $P = 0.003$ ). The results of multiple logistic regression analyses evaluating possible association of skin or nasal symptoms (as dependent variables) with the presence of *FLG* null mutations, controlled for gender, family history of allergic diseases, positive SPT and positive patch test are shown in Table 2. *FLG* null mutations were identified as independent predictor for skin symptoms, while no correlation was found with nasal symptoms. Model with asthma symptoms as a dependent variable was not significant ( $P = 0.091$ , Pseudo  $R^2 = 0.024$ ).

## Discussion

In this study, we observed that only *FLG* null mutation 2282del4 concurs the results of other population-based studies on Caucasians in North America and Western Europe with the prevalence of the stated mutation varying from 2 to 5% (Barnes, 2010;

**Table 1.** Characteristics of subjects with determined FLG null mutations

| Subject | Sex | Mutation | Region of residence | Family history (mother and/or father) |              | Atopic dermatitis | Allergic contact dermatitis | Other dermatitis | Allergic rhinitis and/or asthma |
|---------|-----|----------|---------------------|---------------------------------------|--------------|-------------------|-----------------------------|------------------|---------------------------------|
|         |     |          |                     | Allergy of the respiratory tract      | Skin allergy |                   |                             |                  |                                 |
| 1       | M   | R501X    | C                   | —                                     | —            | —                 | —                           | +                | —                               |
| 2       | F   | 2282del4 | C                   | —                                     | +            | + <sup>a</sup>    | —                           | —                | + <sup>a</sup>                  |
| 3       | F   | 2282del4 | C                   | —                                     | —            | —                 | +                           | —                | —                               |
| 4       | M   | 2282del4 | C                   | —                                     | —            | —                 | —                           | —                | —                               |
| 5       | F   | 2282del4 | C                   | —                                     | —            | —                 | —                           | +                | —                               |
| 6       | F   | 2282del4 | C                   | —                                     | —            | + <sup>a</sup>    | —                           | —                | + <sup>a</sup>                  |
| 7       | M   | 2282del4 | M                   | —                                     | —            | —                 | NA                          | +                | —                               |
| 8       | F   | 2282del4 | M                   | —                                     | —            | —                 | —                           | —                | —                               |
| 9       | M   | 2282del4 | C                   | —                                     | +            | + <sup>b</sup>    | —                           | —                | —                               |
| 10      | F   | 2282del4 | C                   | —                                     | —            | —                 | —                           | +                | —                               |
| 11      | F   | 2282del4 | M                   | —                                     | —            | —                 | —                           | +                | —                               |

Atopic dermatitis: reported skin symptoms during the last 12 months + positive skin prick test to at least one common inhalatory allergen.

Allergic contact dermatitis: reported skin symptoms during the last 12 months + positive patch test to at least one standard contact allergen.

Other dermatitis: reported skin symptoms during the last 12 months + negative patch and prick test.

Skin symptoms: two or more reported symptoms including redness, itching, vesicles and rash.

Allergic rhinitis and/or asthma: reported nasal and asthma symptoms during the last 12 month + positive skin prick test to at least one common inhalatory allergen.

Nasal symptoms: two or more reported symptoms including runny nose, itchy nose, blocked nose and sneezing.

Asthma symptoms: reported wheezing and/or dyspnoea.

<sup>a</sup>Skin prick test positive to house dust mites (*Dermatophagoides pteronyssinus* and/or *D. farinae*); <sup>b</sup>Skin prick test positive to birch and hazel pollen.

NA, not available (patch test was not performed); M, Mediterranean region; C, continental region; FLG, filaggrin.

**Table 2.** Results of multiple logistic regression analysis for allergic skin and respiratory symptoms

|                                                 | Skin symptoms    |        | Nasal symptoms   |         |
|-------------------------------------------------|------------------|--------|------------------|---------|
|                                                 | OR (95% CI)      | P      | OR (95% CI)      | P       |
| Predictors                                      |                  |        |                  |         |
| Gender                                          | 1.66 (0.83–3.34) | 0.152  | 1.15 (0.68–1.92) | 0.603   |
| Family history of allergic disease <sup>a</sup> | 2.78 (1.28–6.03) | 0.010* | 2.97 (1.76–5.04) | <0.001* |
| Positive SPT                                    | 2.21 (1.33–3.67) | 0.002* | 3.12 (1.96–4.98) | <0.001* |
| Positive patch test                             | 1.43 (0.81–2.54) | 0.217  | —                | —       |
| FLG mutation                                    | 17.2 (3.41–86.6) | 0.001* | 1.44 (0.36–5.82) | 0.608   |
| Model                                           |                  |        |                  |         |
| P                                               | <0.001           |        | <0.001           |         |
| Pseudo R <sup>2</sup>                           | 0.087            |        | 0.092            |         |

Results are presented as odds ratio (OR) with 95% confidence interval (95% CI).

\*Significant predictors in the model ( $P < 0.05$ ).

<sup>a</sup>Family history of allergic skin diseases in the model with skin symptoms as dependent variable or family history of respiratory allergic disease in the model with nasal symptoms as dependent variable.

FLG, filaggrin; SPT, skin prick test.

Ponińska *et al.*, 2011). Similarly to the results of recent survey conducted in Poland (Ponińska *et al.*, 2011) and an Italian study (Giardina *et al.*, 2008), the participants of our study showed lower prevalence of

R501X null mutation (less than 1%) in comparison with studies conducted in Irish and Scottish population, which reported so far the highest prevalence of R501X ~6% (Palmer *et al.*, 2006), and German population, which had a relatively intermediate prevalence of R501X ~2.5 (Stemmler *et al.*, 2007; Weidinger *et al.*, 2007). Less common mutations R2447X and S3247X that were present in German cohort (Weidinger *et al.*, 2008) in <1% and showed a relatively higher combined prevalence ~2.5% in United Kingdom and Irish population (Sandilands *et al.*, 2007) were not found among our examined population. Therefore, our results assort to the recent observation that a latitude-dependent distribution exists with a decreasing north–south gradient of R501X and 2282del4 mutation frequencies in Europe (Barnes, 2010). Additionally, the prevalence of the so far studied FLG null mutations coincides with the distribution of ethnical groups in Europe, suggesting a different frequency pattern of R501X and 2282del4 in Slavic populations such as Poland and Croatia in comparison with Germanic populations that reside in United Kingdom, Ireland and Germany (Palmer *et al.*, 2006; Stemmler *et al.*, 2007; Weidinger *et al.*, 2007; Ponińska *et al.*, 2011).

Regarding the observed prevalence of FLG null mutations, it could be concluded that only 2282del4 FLG mutation could be associated with the presence of allergic diseases in Croatian population. Recently, a population-based survey conducted in Croatia reported a 15% prevalence of positive markers of

allergic diseases in adults (Macan *et al.*, 2007), and another study in Croatian schoolchildren based on ISAAC questionnaire established a prevalence of current allergic rhinitis around 11% and of current asthma and atopic dermatitis around 5% (Munivra *et al.*, 2007). Compared to the former observations, our subject group showed higher prevalence of allergic diseases (28%) and this occurrence could possibly be the result of bias as students with allergy-like disorders were more willing to participate in the study. Herein, results indicate that the investigated *FLG* null mutations are not accountable for higher prevalence of allergic disorders in this study group. Additionally, only two *FLG* null mutation carriers had a positive parental history for allergies. Regression model also failed to confirm *FLG* null mutations as predictor for allergic skin diseases. However, these findings should be taken with precaution due to the low frequency of the studied genetic variants and small groups of studied subjects with allergic diseases, which all diminished the power and relevance of statistical analyses. In the studied cohort, atopic dermatitis was present only in 49 subjects, allergic rhinitis in 74 and allergic asthma in 31, and these numbers are far too small to detect the lack of association of *FLG* null mutations and allergic diseases. Moreover, regarding crude risk ratio, *FLG* mutation carriers had 2.6 times higher risk for allergic skin diseases (atopic dermatitis or allergic contact dermatitis) than wild-type carriers confirming previous findings about the relation of *FLG* null mutations and atopic dermatitis. In the published literature, a combined allele frequency of *FLG* null mutations among Northern European patients with atopic dermatitis ranged from 15.8 to 56% (Brown & Irvine, 2008; Ponińska *et al.*, 2011). However, a study conducted in Italian population showed a lack of association of R501X and 2282del4 with atopic dermatitis (Giardina *et al.*, 2008), suggesting that although there is ample evidence of *FLG* null mutations being a predisposing factor for allergic skin diseases, the foundation of these diseases in various populations perhaps reposes on other genetic variants with higher frequency in a specific population.

It was reported that ~40% of all carriers of *FLG* null mutations never develop any signs of allergic skin diseases, which is presumably modified by environmental or genetic modifiers (McGrath, 2008). Our results showed that besides four *FLG* null mutation carriers with allergic skin diseases, five mutation carriers reported having skin symptoms without defined allergic sensitization (positive SPT and/or patch test), and multiple logistic regression model confirmed *FLG* null mutations as independent predictor for presence of skin symptoms. This confirms higher susceptibility of *FLG* mutation carriers' skin to various skin hazards. Regardless of absent allergic sensitization, an abnormal skin barrier function caused by *FLG* null mutations might be sufficient to develop skin symptoms and disorders. An increased susceptibility to

chronic irritant contact dermatitis (ICD) was recently reported to be associated with *FLG* null mutations (OR 1.91; 95% CI 1.02–3.59,  $P = 0.02$ ) (de Jongh *et al.*, 2008). However, the latter study did not distinguish whether *FLG* null mutations contribute to the development of ICD independently of atopic dermatitis presence. In general, atopic dermatitis is considered a predisposing factor for ICD (de Jongh *et al.*, 2008; Davis *et al.*, 2010) and *FLG* null mutations predispose to the development of atopic dermatitis. Therefore, it seems logical to elucidate whether *FLG* null mutations could be a risk factor for ICD independently of atopic dermatitis which our findings suggest.

Previous research showed association of *FLG* null mutations with sensitization to common inhalatory allergens such as grass, HDM (*Dermatophagoides pteronyssinus* and/or *farinae*), cat dander and sensitization to multiple allergens (Henderson *et al.*, 2008; Weidinger *et al.*, 2008). It is suggested that loss of skin barrier function regulated by filaggrin could be implicated in the development of allergic respiratory diseases by allowing allergens to pass into the organism and activate abnormal immune response that fosters rhinitis or airway hyperresponsiveness (Osawa *et al.*, 2011). A direct association is commonly not found between *FLG* null mutations and allergic respiratory diseases in the absence of concomitant atopic dermatitis (Marenholz *et al.*, 2006; Henderson *et al.*, 2008; Vercelli, 2008; Rodríguez *et al.*, 2009; Schuttelaar *et al.*, 2009). Our findings did not differ from these observations, showing no *FLG* null mutation carriers with solely present allergic respiratory diseases, and failing to confirm association of *FLG* null mutations with presence of nasal symptoms. The association with asthma symptoms could not be analysed because of the small number of subjects (56 subjects reported asthmatic symptoms) and therefore low statistical power of the regression model. It should be stated that controversies do exist in this field because German cross-sectional study (Weidinger *et al.*, 2008) conferred *FLG* mutations as a risk factor for allergic rhinitis independently of atopic dermatitis presence (OR 2.64; 95% CI 1.75–4.00;  $P = 2.5 \times 10^{-6}$ ) and Polish cohort revealed an association between *FLG* null variants and allergic asthma in the absence of atopic dermatitis (OR 2.02, 95% CI 1.07–3.81,  $P = 0.027$ ) (Ponińska *et al.*, 2011).

The speculated role of skin barrier filaggrin defect in the development of contact allergy was scarcely investigated so far. We identified 26 subjects (7%) with allergic contact dermatitis, and only one of them was a *FLG* null mutation carrier. Nickel was confirmed as the most frequent cause of allergic contact dermatitis in the general population (Thyssen & Menné, 2010). Up to now, tree studies suggested a risk of developing contact allergy, mainly to nickel, in subjects with *FLG* null mutations (Novak *et al.*, 2008; Thyssen *et al.*, 2010; Ross-Hansen *et al.*, 2011). The results regarding contact allergy in our study should be taken with

caution because generally the prevalence of allergic contact dermatitis seems to be low in young people and development of contact allergy increases with age (Mortz & Andersen, 1999).

In conclusion, our findings in general indicate a low frequency of *FLG* null mutations in studied Croatian population with 2.4% carriers of 2282del4, <1% carriers of R501X and with absence of R2447X or S3247X mutations, supporting the recently presumed theory of a latitude-dependent distribution with a decreasing north–south gradient of R501X and 2282del4 mutation frequencies in Europe (Barnes, 2010) elicited from the study conducted in Italian population (Giardina *et al.*, 2008). Therefore, the frequency of *FLG* null mutations was also low in subjects with allergic diseases including atopic dermatitis, allergic rhinitis and asthma and allergic contact dermatitis (<7%), suggesting that the contribution of the *FLG* null mutations in allergic skin diseases, including atopic dermatitis, depends on the *FLG* mutation frequency in assessed general population. In populations with low penetrance of analysed *FLG* null mutations, possibly some other *FLG* polymorphisms or some other genetic and/or environmental events with influence on allergy status may be dominant. However, we found that *FLG* null mutations increase the risk for skin disorders including allergic skin diseases. Further population-based studies with increased number of participants and other *FLG* polymorphisms may lead to better understanding of the contribution of *FLG* null mutations in allergic diseases in Croatia.

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## Conflict of interest statement

All authors declare no conflict of interest.

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