Coexistence of 2282del4 FLG gene mutation and IL-18 –137G/C gene polymorphism enhances the risk of atopic dermatitis

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Abstract

Introduction: Atopic dermatitis (AD) pathogenesis appears in the context of the correlation between cornified envelope proteins and immunological factors.

Aim: To estimate the association between FLG R501X and 2282del4 gene mutations, –137 G/C IL-18 and –1112 C/T IL-13 gene polymorphisms and their influence on AD course and the risk in the Polish population.

Material and methods: One hundred and fifty-two AD patients and 123 healthy volunteers were included into the study. Amplification refractory mutation system – polymerase chain reaction method was used.

Results: 2282del4 FLG mutation, predominant (p = 0.04) in Polish AD patients, enhanced the risk of AD (OR = 2.35; p = 0.01) and was associated with itch (p = 0.023). GG genotype of IL-18 was prevailing in AD (p < 0.0001), associated with elevated IgE levels (p = 0.00074) and pruritus (p < 0.0001). GG genotype and G-allele in –137 position of IL-18 increased AD risk (OR = 5.4; p = 0.0001, respectively, OR = 5.3; p = 0.000029). –1112 C/T polymorphism of IL-13 was associated with elevated IgE levels (p = 0.00049), pruritus (p = 0.0005), SCORAD score (p = 0.02), concomitant asthma (p = 0.0087) and AD risk (OR = 2.02; p = 0.012). Coexistence of 2282del4 or R501X FLG gene mutation with GG genotype of IL-18 was associated with a 6-fold higher risk of AD (OR = 5.8; p = 0.00013), contrary to combined occurrence of FLG mutations with T-allele in –1112 position of IL-13 gene (OR = 0.12; p = 0.1).

Conclusions: 2282del4 FLG mutation similarly to GG genotype and G-allele in –137 position of IL-18 enhance the risk of AD in the Polish population. Coexistence of FLG mutations with GG genotype of IL-18 may be helpful to estimate chances of AD development.

Key words: filaggrin, interleukin 18, interleukin 13, atopic dermatitis.

Introduction

Atopic dermatitis (AD) is common, chronic inflammatory skin disease [1, 2] with increasing socio-economical influences [3–5]. The clinical picture, the course of the disease, pruritus and sleep disturbances have a significant impact on occupational activity of the patients, worsen their social relations and decrease quality of life of the patients and their families [6]. Atopic dermatitis pathogenesis is multi-factorial. Beside immunological, environmental and genetic factors, the role of the epidermal barrier dysfunction is strongly underlined. Last year researches revealed that in the European population, 2282del4 and R501X filaggrin null mutation are the major predisposing factor in AD development [7–9]. On the other hand, Th2 cytokines like: IL-4, IL-13, IL-25, IL-31 decrease FLG expression even if there is no FLG mutation [10]. Ethnic differences in FLG mutations are also observed [11]. Interleukin 18 (IL-18) is a pleiotropic cytokine, whose activities depend on cytokine milieu and genetic background [12–15]. Recently a key role of this cytokine was raised in allergic diseases. Interleukin 18 induces IgE synthesis by enhancing production of IL-4 and IL-13 [12, 14], but it can also lead to AD-like inflammation in an IgE-independent manner [14, 15]. Interleukin 18 was suggested as a marker of AD severity [12, 16, 17]. The gene encod-
ing IL-18 is located on chromosome 11q22.2–22.3, which has been designated as a candidate region for atopy [12, 13, 18, 19]. The crucial role of interleukin 13 (IL-13) in AD was confirmed by different studies [20–22]. This cytokine is mainly responsible for enhanced IgE levels. Expression of IL-13 correlates with elevated IgE levels and AD severity [23]. It is thought that the polymorphism of IL-13 gene in the promoter region may be related to the increased transcription of that gene and susceptibility to development of AD and increased serum levels of IgE [24].

In the light of recent publications, pathogenesis of AD appears in the context of two major groups of genes: encoding epidermal proteins and major elements of the immune system [2]. Taking this into account, the association between cornified envelope protein gene mutations and cytokine genotyped gene polymorphisms and their influence on AD risk seems to be interesting.

**Material and methods**

Two hundred and seventy-five subjects were included into the study: 152 AD patients diagnosed according to current criteria and 123 healthy volunteers with no previous history of allergic diseases. The M:F (male and female) ratio was 0.8:1. The average age of the AD patients was 23.2±11.57 (age range: 5–56 years) and of the controls was 24.9±8.02 (age range: 8–52 years). Assessment of pruritus severity was performed using the visual analogue scale (VAS: 0–3 – mild pruritus, >3–7 – moderate pruritus, >7–9 – severe pruritus, >9 – very severe pruritus). Atopic dermatitis severity was rated by SCORAD index (severe (SCORAD > 60, pruritus). Atopic dermatitis severity was rated by SCORAD

**Statistical analysis**

The data from inquiry prepared specially for this study were statistically worked out using Excel 2003 (Microsoft Corp., Redmond, WA, USA), Statistica (Version 8.0; StatSoft, Tulsa, OK, USA). The W Shapiro-Wilk, U Mann-Whitney, Kruskal-Wallis and χ² tests were performed. A logistic regression model was used to calculate the odds ratio (OR) and 95% confidence intervals (CIs). The statistical significance was established for the p < 0.05.

**Results**

**Flaggrin**

2282del4 FLG mutation was the predominant one in AD patients with FLG mutations (p = 0.04). We have found 42 (28.6%) patients that were heterozygotes, and no homozygotes (Table 1). 2282del4 of FLG mutation coexisted with allergic rhinitis (p = 0.001) and pruritus (p = 0.03). There was no association of FLG 2282del4 mutation with elevated IgE levels (p = 0.16), early onset of the disease (p = 0.97), concomitant asthma (p = 0.14), SCORAD score (p = 0.97), eosinophilia (p = 0.65). 2282del4 FLG mutation enhanced the risk of AD over twofold (OR = 2.35; p = 0.01) (Table 2).

R501X heterozygous mutation was observed in 20 (13.2%) AD patients (p = 0.18) (Table 1). We have not disclosed any homozygous mutation. There was no association of R501X FLG mutation with pruritus (p = 0.14), elevated IgE levels (p = 0.09), early onset of the disease (p = 0.35), SCORAD score (p = 0.91), eosinophilia (p = 0.21). We have only observed the association of R501X mutation with concomitant asthma (p = 0.0047). R501X mutation had no influence on the AD risk (OR = 0.68; p = 0.38) (Table 2).

The presence of R501X or 2282del4 FLG mutation in AD patients was associated with: pruritus (p = 0.03), elevated IgE levels (p = 0.025), concomitant asthma (p = 0.01) and allergic rhinitis (p = 0.0014). The presence of any of FLG mutations enhanced the risk of AD (OR = 1.88; p = 0.016) (Table 2).

**–137 G/C polymorphism of IL-18**

GG genotype of IL-18 was prevailing in AD (p < 0.0001). It was observed in 94 (61.8%) AD patients (Table 1). GG genotype of IL-18 was dominant in the
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Table 1. The occurrence of FLG mutations, genotypes of IL-18 –137 G/C promoter gene polymorphism and –1112 C/T IL-13 promoter gene polymorphism in atopic dermatitis patients and control group

| Variable | Controls | AD | P-value |
|----------|----------|----|---------|
|          | N = 123  | N = 147 |        |
| 2282del4 FLG mutation | Heterozygotes | 23 (18.7%) | 42 (28.6%) | 0.04 |
|          | Wild type | 100 (81.3%) | 105 (71.4%) | |
| R501X FLG mutation | CT | 10 (8.1%) | 20 (13.2%) | 0.18 |
|          | CC | 113 (91.9%) | 132 (86.8%) | |
| IL-18    | GG | 41 (33.3%) | 94 (61.8%) | < 0.0001 |
|          | GC | 51 (41.5%) | 49 (32.2%) | |
|          | CC | 31 (25.2%) | 9 (5.9%) | |
| IL-13    | CC | 52 (42.3%) | 34 (23.3%) | 0.00028 |
|          | CT | 70 (56.9%) | 102 (69.9%) | |
|          | TT | 1 (0.8%) | 10 (6.8%) | |

Table 2. The influence of FLG mutations, IL-18 and IL-13 genotypes and alleles on the AD risk

| Variable | P-value | OR | –95% CL | +95% CL |
|----------|---------|----|---------|---------|
| 2282del4 FLG mutation | 0.01 | 2.35 | 4.519 | 1.223 |
| R501X FLG mutation | 0.39 | 0.684 | 0.289 | 1.616 |
| 2282del4 or R501X FLG mutations | 0.016 | 1.885 | 3.179 | 1.118 |
| IL-18 | < 0.0001 | 2.672 | 3.861 | 1.849 |
|          | < 0.0001 | 5.412 | 2.761 | 10.608 |
|          | 0.000029 | 5.351 | 2.429 | 11.804 |
|          | < 0.0001 | 0.308 | 0.508 | 0.178 |
| –1112 C/T IL-13 | 0.013 | 2.021 | 1.159 | 3.526 |
| T-allele of IL-13 | 0.001 | 2.319 | 3.940 | 1.365 |
| C-allele of IL-13 | 0.038 | 0.111 | 0.014 | 0.892 |
| 2282del4 or R501X mutation and GG genotype of IL-18 | 0.00013 | 5.815 | 2.342 | 14.439 |
|          | 0.12 | 0.625 | 0.348 | 1.123 |

Group of patients with pruritus in contrast to CC genotype (p < 0.0001). We have observed the association of –137G/C IL-18 polymorphism with elevated IgE levels. GG genotype of IL-18 dominated in the group with enhanced IgE serum concentration comparing to CC genotype that dominated in the group with normal IgE level (p = 0.000074). We have observed an association of –137G/C IL-18 gene polymorphism with allergic rhinitis (p = 0.00027). We have not found any association of –137 G/C polymorphism of IL-18 with early onset of the disease (p = 0.48), concomitant asthma (p = 0.15) or eosinophilia (p = 0.26). –137G/C polymorphism of IL-18 gene enhanced the risk of AD, especially GG genotype (OR = 5.14; p < 0.0001) and G allele (OR = 5.35; p = 0.000029) over 5 times (Table 2).

–1112 C/T polymorphism of IL-13

CT genotype of IL-13 was predominant in AD (69.9%, p = 0.00028) (Table 1). T-allele was dominant in the AD group: 61.2% vs. 38.8% in controls (p = 0.0015). –1112 C/T IL-13 gene polymorphism was associated with pruritus (p = 0.00005) and elevated IgE levels (p = 0.00049),
Discussion

The phenotype of AD is a result of genetic, immunological and environmental influences [25]. Analysis of such a multifactorial disease like AD poses different difficulties. Last year’s research have identified some genes responsible for AD susceptibility and provided a number of valuable clues that let us better understand the genetic background of AD. The FLG mutation is the risk factor for AD in European populations [7–9]. Our data are in accordance with previously reported findings for 2282del4 FLG mutation [8, 9], which was associated with the AD risk in our population, too. In turn, R501X FLG mutation does not contribute to AD in Polish patients, which is in contrast to observations in other European populations such as Irish, British, or German [8, 9]. It was confirmed by most of the authors [7, 11, 26] that carriers of FLG gene mutations manifest a more severe course of AD. This trend was not noted in our group. Similarly to Poninska et al. [27], we have not found any association between 2282del4 FLG gene mutation and elevated serum IgE levels, what was in contrast to others [7–9, 26]. These differences may be explained by effect size or ethnic limitations. We have not observed any association of 2282del4 of FLG gene mutation with early onset of AD like Lesiak et al. [28], but some researches in European and Asian populations have indicated such a correlation [7, 11, 26].

Similarly to our studies, in the Danish population [29], a relationship between a polymorphic variant of the IL-13 gene and AD occurrence was indicated. In the Japanese population [30], significant differences in the frequency of each allele and genotype of the IL-13 gene between AD patients and healthy subjects were not found, in spite to our data. The association of IL-13 polymorphisms with the increased serum IgE levels was demonstrated in a group of 1399 children with atopic diseases [31] and in the German population [32], as well as in our results. On the other hand, the scientists at several European centers studied a group of 453 AD children and did not demonstrate any associations between polymorphism in the promoter region of IL-13 and the IgE level for the TT homozygotes, while they observed slightly increased IgE levels for the CT heterozygotes [33].

Results of our study are consistent with the the one carried out in the German population [13], which revealed a significant association of SNPs in –137G/C of IL-18 gene with AD. We have previously published the data, which have indicated that G-allele reveals susceptibility to AD development and C-allele seems to have protective properties [34]. Now we have more proofs because GG genotype of IL-18 is associated with elevated IgE levels and pruritus in contrast to CC genotype. Kruse et al. [19] have also observed an association of –137 G/C IL-18 gene polymorphism with high IgE levels. By the way, we have found no association with SCORAD score, similarly to Novak et al. [13]. According to the latter study, the association of –137 G/C SNP of IL-18 with AD was not directly dependent on concomitant manifestation of allergic rhinitis or asthma. We have noticed the associations of –137 G/C SNP of IL-18 gene polymorphism with concomitant allergic rhinitis, but not with asthma. The association of IL-18 serum levels with AD course was previously published [16, 17]. Now, in the context of FLG mutations, we have found no associations between elevated serum levels of IL-18 and 2282del4 FLG mutation as well as for R501X FLG
mutation. Anyway, it is well documented that Th2 cytokines like IL-13, IL-4 influence the FLG expression even if no FLG mutation exists [10]. It seems to be interesting if IL-18 also inhibits FLG expression in AD patients, while they are not FLG mutation carriers. On the other hand, in our study AD 2282del4 carriers with homozygous GG genotype for −137 G/C polymorphism of IL-18 have a nearly 6-fold higher risk of AD development. We have previously published that elevated levels of IL-18 were associated with GG genotype and G allele [34]. Additionally, although −1112 C/T polymorphism of IL-13 gene and T allele enhance the risk of AD according to previous data [35], coexistence of T allele with any FLG mutation does not increase susceptibility to AD.

These results seem to indirectly indicate that there must be an interaction between FLG decreased expression and Th2 cytokines over-expression, as it was previously suggested [4, 36] and AD emerges in the light of innate and acquired immune response [37], genes and immunology interactive net.

Conclusions

2282del4 FLG mutation that dominates in the Polish population is a risk factor for AD development. GG genotype and G allele of IL-18, similarly to T allele of IL-13, seem to promote AD development. In contrast to combined occurrence of FLG mutations with T allele of IL-13, coexistence of FLG mutations with GG genotype of IL-18 is associated with a 6-fold higher risk of AD. Thus, our results indicate that this combined occurrence may be helpful to estimate chances of AD development and seems to be a useful parameter in separating patients from healthy persons.

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Conflict of interest

The authors declare no conflict of interest.

References

1. Boguniewicz M, Leung DY. Atopic dermatitis: a disease of altered skin barrier and immune dysregulation. Immunol Rev 2011; 242: 233-46.
2. Bieber T. Atopic dermatitis. N Eng J Med 2008; 358: 1483-4.
3. Wollesenberg A, Bieber T. Proactive therapy of atopic dermatitis – an emerging concept. Allergy 2009; 64: 276-8.
4. Rutkowski K, Sowa P, Rutkowska-Talipska J, et al. Allergic diseases: the price of civilizational progress. Adv Dermatol Allergol 2014; 31: 77-83.
5. Sybilski A, Rakowksi F, Lipiec A, et al. Atopic dermatitis is a serious health problem in Poland. Epidemiology studies based on the ECAP study. Adv Dermatol Allergol 2015; 32: 1-10.
6. Warschburger P, Buchholz H, Petermann F. Psychological adjustment in parents of young children with atopic der- matitis: which factors predict parental quality of life? Br J Dermatol 2004; 150: 304-11.
7. Palmer CN, Irvine AD, Terron-Kiwiakowski A, et al. Common loss-of-function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis. N At Genet 2006; 38: 441-6.
8. Van den Oord RA, Sheikh A. Filaggrin gene defects and risk of developing allergic sensitisation and allergic disorders: systematic review and meta-analysis. BMJ 2009; 339: b2433.
9. Rodriguez E, Baurecht H, Herberich E, et al. Meta-analysis of filaggrin polymorphisms in eczema and asthma: robust risk factors in atopic disease. J Allergy Clin Immunol 2009; 123: 1361-70.
10. Howell MD, Kim BE, Gao P, et al. Cytokine modulation of atopic dermatitis filaggrin skin expression. J Allergy Clin Immunol 2007; 120: 150-5.
11. Chen H, Common JE, Haines RL, et al. Wide spectrum of filaggrin-null mutations in atopic dermatitis highlights differences between Singaporean Chinese and European populations. BJ Dermatol 2011; 165: 106-14.
12. Kim E, Lee JE, Namkung JH, et al. Association of the single nucleotide polymorphism and haplotype of the interleukin 18 gene with atopic dermatitis in Koreans. Clin Exp Allergy 2007; 37: 865-71.
13. Novak N, Kruse S, Potreck J, et al. Single nucleotide polymorphisms of the IL-18 gene are associated with atopic eczema. Allergy Clin Immunol 2005; 115: 828-33.
14. Yoshimoto T, Tsutsui H, Tominaga K, et al. IL-18, although antiallergic when administrated with IL-12, stimulates IL-4 and histamine release by basophils. Proc Natl Acad Sci USA 1999; 24: 1362-6.
15. Konishi H, Tsutsui H, Murakami T, et al. IL-18 contributes to the spontaneous development of atopic dermatitis-like inflammatory skin lesion independently of IgE/stat6 under specific pathogen-free conditions. Proc Natl Acad Sci USA 2002; 17: 11340-5.
16. Trzeciak M, Gleb J, Bandurski T, et al. Relationship between serum levels of interleukin-18, IgE and disease severity in patients with atopic dermatitis. Clin Exp Dermatol 2011; 36: 728-32.
17. Kou K, Aihara M, Matsunaga T, et al. Association of serum interleukin-18 and other biomarkers with disease severity in adults with atopic dermatitis. Arch Dermatol Res 2012; 304: 305-12.
18. Koppelman GH, Stine OC, Xu J, et al. Genome-wide search for atopy susceptibility genes in Dutch families with asthma. J Allergy Clin Immunol 2002; 109: 498-506.
19. Kruse S, Kuehr J, Moseler M, et al. Polymorphisms in the IL-18 gene with atopic dermatitis in Koreans. Clin Exp Immunol 2005; 137: 117-22.
20. Brandt EB, Sivaprasad U. Th2 cytokines and atopic dermatitis. J Clin Cell Immunol 2011; 2: 110.
21. Zheng T, Oh MH, Oh SY, et al. Transgenic expression of interleukin-13 in the skin induces a pruritic dermatitis and skin remodeling. J Invest Dermatol 2009; 129: 742-51.
22. Hamid Q, Boguniewicz M, Leung DY. Differential in situ cytokine expression in acute versus chronic atopic dermatitis. J Clin Invest 2006; 94: 870-6.
23. Metwally SS, Mosaad YM, Abdel-Samee ER, et al. IL-13 gene expression in patients with atopic dermatitis: relation to IgE level and to disease severity. Egypt J Immunol 2004; 11: 171-7.

24. Soderhall C, Bradley M, Kockum I, et al. Linkage and association to candidate regions in Swedish atopic dermatitis families. Hum Genet 2003; 109: 129-35.

25. Cork MJ. J Epidermal barrier dysfunction in atopic dermatitis. In: Dermatol 2009; 129: 1892-908.

26. Weidinger S, Rodríguez E, Stahl C, et al. Filaggrin mutations strongly predispose to early-onset and extrinsic atopic dermatitis. J Invest Dermatol 2007; 127: 724-6.

27. Poninska J, Samoliński B, Tomaszewska A, et al. Filaggrin gene defects are independent risk factors for atopic asthma in a Polish population: a study in ECAP cohort. PLoS One 2011; 6: e16933.

28. Lesiak A, Kuna P, Zakrzewski M, et al. Combined occurrence of filaggrin mutations and IL-10 or IL-13 polymorphisms predisposes to atopic dermatitis. Exp Dermatol 2011; 20: 491-5.

29. Hummelshøj T, Bodtger U, Datta P, et al. Association between an interleukin-13 promoter polymorphism and atopy. Eur J Immunogenet 2003; 30: 355-9.

30. Tsunemi Y, Saeki H, Nakamura K, et al. Interleukin-13 gene polymorphism G4257A is associated with atopic dermatitis in Japanese patients. J Dermatol Sci 2002; 30: 100-7.

31. Graves PE, Kabesch M, Halonen M, et al. A cluster of seven tightly linked polymorphisms in the IL-13 gene is associated with total serum IgE levels in three populations of white children. J Allergy Clin Immunol 2000; 105: 506-13.

32. Liu X, Nickel R, Beyer K, et al. An IL-13 coding region variant is associated with a high total serum IgE level and atopic dermatitis in the German multicenter atopy study (MAS-90). J Allergy Clin Immunol 2000; 106: 167-70.

33. Zitnik SE, Rüschendorf F, Müller S, et al. IL13 variants are associated with total serum IgE early sensitization to food allergens in children with atopic dermatitis. Pediatr Allergy Immunol 2009; 20: 551-5.

34. Trzeciak M, Gleń J, Roszkiewicz J, et al. Association of interleukin-18 SNP with atopic dermatitis. J Eur Acad Dermatol Venereol 2010; 24: 78-9.

35. Gleń J, Trzeciak M, Sobjanek M, et al. Interleukin-13 promoter gene polymorphism -1112 C/T is associated with atopic dermatitis in Polish patients. Acta Dermatovenerol Croat 2012; 20: 231-8.

36. Leung DY. Our evolving understanding of the functional role of filaggrin in atopic dermatitis. J Allergy Clin Immunol 2009; 124: 494-5.

37. O’Regan GM, Sandilands A, McLean WH, et al. Filaggrin in atopic dermatitis. J Allergy Clin Immunol 2008; 122: 689-93.