

Association between Loss-of-Function Mutations in the Filaggrin Gene and Self-Reported Food Allergy and Alcohol Sensitivity

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Key Words

Filaggrin · Alcohol sensitivity · Food hypersensitivity · Lactose intolerance · Oral allergy syndrome

Abstract

Background: Loss-of-function mutations of the filaggrin (FLG) gene cause an impaired skin barrier and increase the risk of atopic dermatitis. Interestingly, FLG mutations have also been found to be associated with a high risk of peanut allergy. **Objective:** We investigated the association of FLG mutations with self-reported food allergy, symptoms of oral allergy syndrome (OAS), and alcohol sensitivity. **Methods:** A total of 3,471 adults from the general population participated in a health examination. Information on food allergies, OAS and alcohol sensitivity was obtained by questionnaire. FLG mutation carriers were defined as having at least one null mutation allele of R501X or 2282del4. Primary lactose intolerance (PLI) was defined as the C/C genotype of the rs4988235 polymorphism. **Results:** FLG mutations were associated with a higher risk of self-reported allergy to eggs (OR 3.22 and 95% CI 1.46–7.11), milk (OR 2.10 and 95% CI 1.12–

3.92), fish (OR 4.54 and 95% CI 1.88–10.96) and wheat (OR 3.59 and 95% CI 1.61–8.02), but not with symptoms of OAS (OR 1.05 and 95% CI 0.73–1.51). Serum-specific IgE was measured in a subsample and confirmed the association between FLG and IgE to milk. A significant gene-by-gene interaction between FLG and PLI was observed in relation to self-reported allergy to milk. Furthermore, FLG mutations were associated with a higher risk of alcohol sensitivity. **Conclusions:** We found that loss-of-function mutations in the FLG gene were significantly associated with self-reported food allergy and alcohol sensitivity, but not with OAS. These findings, if confirmed, support the idea that skin barrier functions may be involved in the pathogenesis of food allergy.

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Introduction

The filaggrin (FLG) protein is crucial to the barrier function of the skin. It is transcribed from the FLG gene on chromosome 1q21 residing in the epidermal differentiation complex [1]. A deletion, 2282del4, and a nonsense

mutation, R501X, both common in Caucasian populations, result in loss of FLG production and are thus functional null alleles for FLG [2] causing a dysfunctional skin barrier [3–7]. The FLG protein is expressed in the outer layers of the epidermis where it aggregates keratin filaments to maintain the barrier function. Moreover, breakdown products of the FLG protein, known as natural moisturizing factors, have important functions like maintaining skin hydration and decreasing skin-surface pH, increasing resistance to skin infections and protecting against UV radiation from the sun. The FLG mutations are a causative factor for ichthyosis vulgaris [2] and a major risk factor for atopic dermatitis [8]. In recent meta-analyses, the presence of FLG null mutations has been confirmed not only as a risk factor for atopic eczema, but also for allergic sensitization to inhalant allergens, allergic rhinitis and asthma in individuals with atopic eczema [9, 10]. While dry skin and a disrupted skin barrier represent relatively obvious links between FLG mutations and atopic dermatitis, the biological mechanisms underlying the association between FLG mutations and respiratory diseases are much less evident as FLG is apparently not expressed in the respiratory mucosa [11, 12], although FLG transcripts have indeed been found in the trachea [13]. It has been proposed that the skin barrier disruption may facilitate allergen penetration through the skin and subsequently promote Th2 deviation of the immune response, IgE sensitization and subsequently asthma [14–17]. Of potential relevance, we have recently reported that smoking modified the effect of FLG null mutations on the risk of asthma, suggesting that interactions between skin and environment are important [18].

The fact that food allergy and atopic dermatitis are strongly associated may warrant investigations of common susceptibility genes. To our knowledge, there is only one published study on the relationship between FLG mutations and food allergy. The study showed that FLG null mutations were associated with peanut allergy defined by food challenge or a combination of history and IgE sensitization to peanut [19]. This association was remarkably strong (an approximately 5-fold relative risk) and was replicated in an independent population. This and other findings raise the question whether sensitization to at least some foods can occur through the skin. However, peanut allergy is unique because skin exposure may occur when using ointments containing peanut oil. It is not known whether allergies to other food items such as wheat, milk, and eggs are associated with FLG mutations. It is also not known whether food-allergic reactions that are generally considered to be due to cross-reactivity

between homologous food and inhalant allergens (e.g. birch or grass), also known as the oral allergy syndrome (OAS), are related to FLG mutations. The OAS is different from food allergy in that the airways represent the likely route of primary sensitization.

Alcoholic drinks are less recognised as elicitors or co-factors of food-allergic reactions than other food items [20]. However, alcoholic drinks are common triggers of sensitivity symptoms from the airways and skin, and people with allergic rhinitis or asthma seem to be at particular risk of alcohol sensitivity reactions [21, 22]. The mechanisms underlying alcohol sensitivity reactions are not fully understood, but IgE-mediated mechanisms are likely not important. The observation that a genetically determined fast metabolism of ethanol to acetaldehyde is associated with an increased risk of alcohol sensitivity [23] lends support to the hypothesis that acetaldehyde can destabilize mast cells, thereby increasing the risk of sensitivity reactions [24]. It may also be relevant that alcohol increases the permeability of the gastrointestinal tract, thereby facilitating the penetration of food proteins through the intestinal mucosal barrier.

In a general population of adults, we aimed to investigate the association of common loss-of-function mutations in the FLG gene with self-reported food allergy, OAS and alcohol sensitivity.

Materials and Methods

Study Population

A sample of 7,931 Danish citizens 18–69 years of age, born in Denmark and living in one of 11 municipalities of Copenhagen, the capital, was randomly drawn from the Danish Civil Registration System and invited to participate in a general health examination, the Health 2006 [25]. A total of 3,471 (43.8%) individuals of Caucasian origin were included in the study and examined between June 2006 and June 2008. All participants completed a questionnaire on lifestyle, health and socioeconomic factors. Young age and male sex were associated with nonparticipation ($p < 0.001$) [25].

Definition of Variables from the Questionnaire

Information about food allergies, OAS and alcohol sensitivity was obtained by a questionnaire which was mailed to participants and completed at home.

Food allergy was defined by using the following questions: 'Have you ever had any allergies/sensitivities to foods?' If 'yes', they were asked to tick one or more of the following food items: 'milk', 'eggs', 'fish' and 'wheat'.

OAS was defined as a positive answer to the question: 'Have you ever had itching in your mouth or throat, or on your tongue or lips following intake of specific food items?' If 'yes', they were asked to tick one or more on a list of food items (shown in table 1).

Alcohol sensitivity was assessed using questions previously employed in a general population survey [22]. It was defined as a positive answer to at least one of the following 3 questions. (1) Have you ever experienced itchy nose, sneezing, runny nose or stuffy nose following intake of alcoholic drinks? (2) Have you ever experienced wheezing or whistling in your chest, breathlessness, or coughing following intake of alcoholic drinks? (3) Have you ever experienced symptoms such as a rash, itching or swelling of the skin, following intake of alcoholic drinks?

Information on alcohol consumption was obtained from the questionnaire. The average amount and the type (beer, wine, dessert wine and spirits) of alcoholic beverage consumed per week in the last 12 months were reported. A standard drink was defined as 1.5 cl or 12 g of pure ethanol. Thus, 1 beer, 1 glass of wine or 1 glass of spirit was considered as 1 standard drink. The total weekly alcohol intake was calculated as the sum of weekly intakes of beer, wine, dessert wine and spirits. Alcohol consumption was categorized into nondrinkers and light (1–7 drinks per week), moderate (7.5–14 drinks per week) and heavy (>14 drinks per week) drinkers.

Questions on asthma were adopted from the stage 1 questionnaire of the European Community Respiratory Health Study [26]. Asthma was defined according to Sunyer et al. [27] as an affirmative answer to at least 1 of the following 3 questions. (1) Have you been woken by an attack of shortness of breath at any time in the last 12 months? (2) Have you had an attack of asthma in the last 12 months? (3) Are you currently taking any medicine (including inhalers, aerosols or tablets) for asthma?

Hay fever was defined as a combination of self-reported symptoms of rhinitis following exposure to allergens and atopy (see below).

Atopic dermatitis was defined according to the UK Working Party's criteria [28] as a history of an itchy skin condition plus a minimum of 2 of 4 minor criteria: (1) a history of flexural involvement, (2) a history of generalised dry skin, (3) onset of rash before the age of 2 years and (4) a history of asthma/hay fever [29].

Finally, participants were divided into 5 age groups (≤ 30 , 31–40, 41–50, 51–60 and >60 years).

Measurements of Serum-Specific IgE to Food Allergens in Subsample

To confirm the association between FLG mutations and food allergy, we analyzed serum-specific IgE against milk, wheat and codfish using the ImmunoCAP, in a subsample of 1,232 consecutively examined participants. The ImmunoCAP Specific IgE is a quantitative enzyme fluorescence immunoassay based on a cellulose derivate solid phase, with very high allergen binding capacity to ensure binding of all relevant antibodies regardless of antibody affinity. Serum-specific IgE positivity was defined as values ≥ 0.35 kU.

Genotyping

Genomic DNA was obtained from 3,366 participants (97.0%). Regions covering the two most common null mutations of the FLG gene, R501X and 2282del4, were amplified from genomic DNA by PCR. The obtained DNA fragments were hybridized to both mutation-specific and wild-type-specific probes attached to paramagnetic beads (Luminex, Austin, Tex., USA) and subsequently analyzed on a BioPlex 200 (Biorad, Hercules, Calif., USA). The genotyping success (call) rates were 99.4 and 99.5% for the

R501X and 2282del4 mutations, respectively, and a total of 3,346 individuals (96.4% of the total study population) were genotyped successfully for both mutations. Individuals were dichotomized into homozygote wild type or carriers of at least one null mutation. None of the observed genotype frequencies deviated significantly ($p > 0.05$) from the expected frequencies under assumption of the Hardy-Weinberg equilibrium. In additional analyses, we examined a gene-dose effect of the number of FLG null alleles at the two FLG loci by defining 3 categories: 'no null mutations', 'one null mutation allele (heterozygous at only one loci)' or 'two or more null mutation alleles at the two loci (homozygous at one locus or compound heterozygous at the two loci)'.

People were, in addition, genotyped for primary lactose intolerance (PLI; –13910 C/T, rs4988235). This SNP was genotyped by KBiosciences allele-specific PCR (KASPar, KBiosciences, Hoddesdon, UK). The call rate was 99.0% with an error rate of 0.0% in 367 duplicate samples. The observed genotype frequencies did not deviate significantly ($p > 0.05$) from the expected frequencies under assumption of the Hardy-Weinberg equilibrium. PLI was defined as the presence of the C/C genotype of the rs4988235 polymorphism [30].

Statistical Analyses

The Hardy-Weinberg equilibrium was calculated using exact tests with GENEPOP v4.0.10 [31]. Crude associations, expressed as odds ratios (ORs) with 95% confidence intervals (CIs) of FLG null mutations with self-reported food allergy, OAS and prevalence of alcohol sensitivity, were analyzed using logistic regression analyses. In additional analyses, ORs were adjusted for sex, age-group and atopic dermatitis. Test for interactions was performed by the likelihood ratio test. Nondrinkers were excluded from the analyses of alcohol sensitivity.

Ethics

The study was approved by the Danish Data Protection Agency and the local ethics committee (KA-2006–0011).

Results

The overall prevalence of self-reported food allergy (to at least one of eggs, milk, fish or wheat) and OAS was 4.2% (95% CI 3.5–4.8%) and 13.2% (95% CI 12.1–14.3%), respectively. FLG null mutations were significantly associated with food allergy to eggs, milk, fish, wheat and at least one of the 4 food items (table 1). These associations remained statistically significant after adjustment for sex, age and atopic dermatitis. There were no statistically significant interactions between FLG mutations and atopic dermatitis in relation to any food-allergic outcome. In addition, the prevalence of self-reported food allergy to eggs and milk, but not to fish and wheat, increased with the increasing number of FLG null mutation alleles, indicating a gene-dose effect (table 2). The associations between self-reported food allergy (to at least one of eggs, milk, wheat and fish) and FLG mutations (shown in table 1)

Table 1. Prevalence of self-reported food allergies/sensitivities according to the FLG genotype

	FLG wild-type % (n/total n)	FLG mutation carriers ¹ % (n/total n)	Crude OR (95% CI)	Adjusted OR (95% CI) ²
Food allergy to				
Egg	1.0 (29/3050)	3.0 (8/267)	3.22 (1.46–7.11)	2.51 (1.11–5.66)
Milk	2.2 (67/3050)	4.5 (12/267)	2.10 (1.12–3.92)	2.00 (1.06–3.81)
Fish	0.6 (18/3050)	2.6 (7/267)	4.54 (1.88–10.96)	2.98 (1.14–7.74)
Wheat	0.9 (26/3050)	3.0 (8/267)	3.59 (1.61–8.02)	2.87 (1.21–6.82)
At least one of above	3.8 (117/3050)	9.0 (24/267)	2.48 (1.56–3.92)	2.13 (1.32–3.44)
OAS following intake of				
Apple	4.1 (124/3043)	3.4 (9/266)	0.82 (0.41–1.64)	0.68 (0.34–1.64)
Peach	2.2 (67/3043)	3.4 (9/266)	1.56 (0.77–3.16)	1.29 (0.63–2.66)
Kiwi	4.8 (145/3043)	4.5 (12/266)	0.94 (0.52–1.73)	0.74 (0.40–1.39)
Pineapple	4.0 (123/3043)	4.5 (12/266)	1.12 (0.61–2.06)	0.91 (0.48–1.72)
Strawberry	0.9 (26/3043)	1.5 (4/266)	1.77 (0.61–5.12)	1.51 (0.51–4.51)
Celery	0.3 (10/3043)	0.8 (2/266)	2.30 (0.50–10.54)	2.08 (0.43–9.98)
Hazelnut	6.1 (187/3043)	5.6 (15/266)	0.91 (0.53–1.57)	0.73 (0.42–1.28)
Orange	1.5 (46/3043)	2.6 (7/266)	1.76 (0.79–3.94)	1.52 (0.66–3.50)
Tomato	3.3 (101/3043)	6.0 (16/266)	1.86 (1.08–3.21)	1.67 (0.95–2.94)
At least one of above	13.0 (396/3043)	13.5 (36/266)	1.05 (0.73–1.51)	1.11 (0.76–1.63)
Alcohol sensitivity ³				
Upper airway symptoms	4.9 (127/2607)	8.3 (19/229)	1.77 (1.07–2.92)	1.74 (1.04–2.89)
Lower airway symptoms	1.0 (27/2605)	3.5 (8/229)	3.46 (1.55–7.70)	2.79 (1.22–6.35)
Skin symptoms	4.0 (103/2602)	5.2 (12/229)	1.34 (0.73–2.48)	1.29 (0.69–2.41)
At least one of above	8.6 (223/2597)	13.1 (30/229)	1.61 (1.07–2.41)	1.56 (1.03–2.35)
Other characteristics				
Female gender	55.0 (1692)	58.1 (157/270)	1.14 (0.88/1.46)	1.06 (0.82–1.38)
Alcohol: nondrinker	14.1 (429/3040)	13.6 (36/265)	0.96 (0.66–1.38)	0.89 (0.61–1.30)
Current smoker	22.4 (682/3046)	25.7 (69/268)	1.20 (0.90–1.60)	1.21 (0.90–1.61)
Atopic dermatitis	9.0 (269/2989)	21.3 (56/263)	2.74 (1.99–3.77)	2.70 (1.95–3.74)
Asthma	10.6 (322/3027)	16.5 (44/266)	1.67 (1.18–2.35)	1.49 (1.05–2.12)
Hay fever	16.8 (206/3017)	18.7 (50/267)	1.14 (0.83–1.58)	0.94 (0.97–1.32)
PLI ⁴	5.5 (169/3051)	6.3 (17/269)	1.15 (0.69–1.93)	1.23 (0.73–2.08)

Figures in bold type represent statistically significant associations.

¹ FLG null mutation carriers defined as at least one null mutation allele of R501X or 2282del4.

² OR adjusted for sex, age-group (18–41, 41–55, 55+ years) and atopic dermatitis.

³ Nondrinkers excluded from the analyses. Alcohol sensitivity was defined as at least one of the following. (1) Upper airway symptoms: 'Have you ever experienced itchy nose, sneezing, runny nose or stuffy nose following intake of alcoholic drinks?'. (2) Lower airway symptoms: 'Have you ever experienced wheezing or whistling in your chest, breathlessness, or coughing following intake of alcoholic drinks?'. (3) Skin symptoms: 'Have you ever experienced symptoms such as a rash, itching or swelling of the skin following intake of alcoholic drinks?'.
⁴ PLI was defined by the presence of the CC genotype of the rs4988235 polymorphism.

were essentially unchanged after further adjustment for 'self-reported hand eczema ever'. There was no statistically significant interaction between FLG mutations and 'self-reported hand eczema ever' in relation to self-reported food allergy.

In contrast, there were no statistically significant associations between FLG null mutations and the symp-

toms of OAS (table 1). Since PLI may be misclassified as allergy to milk, we examined whether the association between FLG mutations and food allergy was dependent on PLI as defined by the CC genotype of the rs4988235 polymorphism (table 3). As expected, the prevalence of self-reported allergy to milk was significantly higher among individuals with PLI than among those without (6.5 vs.

Table 2. The association of self-reported food allergy, OAS and alcohol sensitivity according to the number of FLG mutation alleles

	No FLG mutation alleles % (n/total n)	1 FLG mutation allele ¹ % (n/total n)	2 or more FLG mutation alleles ² % (n/total n)	p value χ^2 / χ^2 for trend
Food allergy to				
Egg	1.0 (29/3050)	2.7 (7/258)	11.1% (1/9)	0.001/0.001
Milk	2.2 (67/3050)	4.3 (11/258)	11.1% (1/9)	0.026/0.010
Fish	0.6 (18/3050)	2.7 (7/258)	0% (0/9)	0.001/n.a.
Wheat	0.9 (26/3050)	3.1 (8/258)	0% (0/9)	0.003/n.a.
At least one of above	3.8 (117/3050)	8.5 (22/258)	22.2% (2/9)	<0.001/<0.001
OAS ³	13.0 (396/3043)	13.2 (34/257)	22.2% (2/9)	0.713/0.705
Alcohol sensitivity ⁴				
Upper airway symptoms	4.9 (127/2607)	8.1 (18/223)	16.7% (1/6)	0.051/0.018
Lower airway symptoms	1.0 (27/2605)	3.6 (8/223)	0% (0/6)	0.004/n.a.
Skin symptoms	4.0 (103/2602)	5.4 (12/223)	0% (0/6)	0.517/n.a.
At least one of above	8.6 223/2597	13.0 (29/223)	16.7% (1/6)	0.069/0.021

n.a. = Trend test not applicable.

¹ One null mutation allele of R501X or 2282del4 (heterozygous at only one locus).

² Two or more mutation alleles of R501X or 2282del4 (homozygous at one locus or compound heterozygous at both loci).

³ Itching in mouth or throat or on tongue or lips following intake of apple, peach, kiwi, pineapple, strawberry, celery, hazelnut, orange or tomato.

⁴ Nondrinkers excluded from the analyses. Alcohol sensitivity was defined as at least one of the following. (1) Upper airway symptoms: 'Have you ever experienced itchy nose, sneezing, runny nose or stuffy nose following intake of alcoholic drinks?'. (2) Lower airway symptoms: 'Have you ever experienced wheezing or whistling in your chest, breathlessness, or coughing following intake of alcoholic drinks?'. (3) Skin symptoms: 'Have you ever experienced symptoms such as a rash, itching or swelling of the skin, following intake of alcoholic drinks?'.

2.1%, χ^2 test, p-value < 0.001). In contrast, PLI was not significantly associated with other food allergies (eggs, fish or wheat), OAS or alcohol sensitivity (data not shown). Interestingly, a statistically significant (p = 0.006) gene-by-gene interaction was observed, i.e. the effect of FLG mutations on the risk of self-reported allergy to milk was dependent on the genetically determined PLI (table 3). Thus, in subjects with PLI, FLG loss-of-function mutations were associated with a 10-fold increased risk of self-reported allergy to milk, while there was no association between FLG mutations and milk allergy in subjects without PLI (table 3).

In a subsample of 1,232 consecutively examined participants, we analyzed serum-specific IgE against milk, wheat and codfish. The results showed that serum IgE positivity to these allergens was higher among FLG mutation carriers than among noncarriers, although this was only statistically significant for sIgE positivity to milk (table 4).

In alcohol drinkers, the prevalence of self-reported upper or lower airway symptoms following the intake of alcoholic drinks was significantly associated with FLG null

mutations (table 1) and the prevalence of upper airway symptoms following the intake of alcoholic drinks increased with an increasing number of FLG null mutation alleles (table 2). We found a statistically significant interaction between FLG genotype and alcohol consumption in relation to alcohol sensitivity (p = 0.014) (fig. 1). The OR for reporting alcohol sensitivity for individuals with FLG null mutations compared to wild-type carriers was 0.92 (p = 0.792) for individuals drinking between 1 and 7 standard drinks per week, 2.57 (p = 0.019) for 8–14 drinks per week and 2.81 (p = 0.004) for more than 14 drinks per week. In individuals reporting the drinking of alcohol within the last 12 months, the prevalence of FLG mutations was 7.7, 10.6, 16.1 and 33.3% in individuals reporting 0, 1, 2 and 3 of the alcohol sensitivity symptoms, respectively (p = 0.004, test for trend). The association between alcohol sensitivity and FLG genotype was independent of asthma, hay fever and atopic dermatitis (p values for tests for interaction all above 0.3).

There was no significant association of alcohol consumption with atopy or FLG mutations (data not shown). People with asthma, hay fever, food allergy and atopic

Table 3. The associations (expressed as ORs with 95% CIs) between self-reported food allergy and loss-of-function FLG mutations stratified by PLI

	PLI		p value for interaction*
	yes	no	
Food allergy to			
Egg	11.00 (0.66–184.87)	3.02 (1.30–7.01)	0.390
Milk	10.33 (2.81–37.89)	1.17 (0.50–2.73)	0.006
Fish	NA	3.88 (1.53–9.87)	NA
Wheat	5.47 (0.47–63.86)	3.40 (1.45–7.98)	0.721
At least one of above	8.98 (2.51–32.08)	1.98 (1.18–3.31)	0.031

FLG null mutation carriers were defined as at least one null mutation allele of R501X or 2282del4. PLI was defined by the presence of the CC genotype of the rs4988235 polymorphism. n.a. = Not applicable (not possible to calculate OR due to empty cell in cross-tabulation).

* p value for test for interaction between loss-of-function FLG mutations and PLI in relation to self-reported food allergy.

Table 4. The prevalence of serum-specific IgE positivity (> 0.35 kU) to milk, wheat and codfish stratified by FLG mutation status in a subsample of the study population

Serum-specific IgE	FLG wild-type % (n/total n)	FLG mutation carriers % (n/total n)	Crude OR (95% CI)
sIgE to milk	1.2 (15/1232)	3.9 (4/103)	3.23 (1.07–10.07)
sIgE to wheat	2.3 (28/1232)	3.9 (4/103)	1.74 (0.60–5.05)
sIgE to codfish	0.2 (3/1232)	1.0 (1/103)	4.02 (0.41–38.96)

FLG null mutation carriers defined as at least one null mutation allele of R501X or 2282del4.

dermatitis reported drinking less alcohol than the people without these conditions, but this was only statistically significant for food allergy and atopic dermatitis (data not shown).

Discussion

In a general adult population, we found that loss-of-function mutations in the FLG gene were significantly associated with self-reported food allergy and alcohol sensitivity, but not with OAS.

The observation that FLG mutations are a strong risk factor for food allergy is difficult to reconcile with the notion that FLG proteins are only expressed in the skin and probably not in the gastrointestinal mucosa. There is some evidence that FLG-deficient skin is more susceptible to the penetration of and sensitization to inhalant al-

lergens [3, 14–17]. It is unknown whether this also relates to food allergens. It is thus possible that the skin can function as the route of primary sensitization to foods in people with a genetically impaired skin barrier function. This would also explain why some cases of food allergy appear to develop in individuals who deny previous oral intake of the offending food item [32]. There is evidence that the introduction of high doses of allergens to the immune system, like when food allergens are ingested, may favor the development of immunological tolerance, while the introduction of low doses of allergens, like when allergens are inhaled, favors sensitization [33]. It may thus be hypothesized that the very low dose of food allergen exposure in the skin, in contrast to the very high dose of food allergen exposure that occurs when allergens are ingested, may promote sensitization. Alternatively, FLG may be hypothesized to increase the risk of sensitization to food allergens, if FLG has a role in maintaining bar-

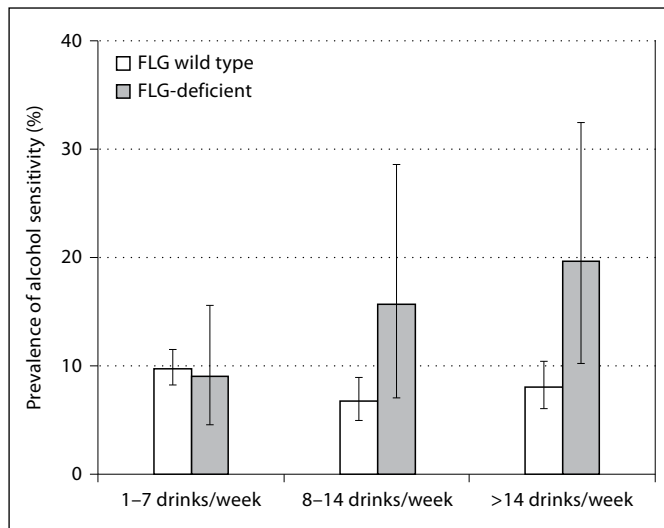


Fig. 1. Interaction between the FLG null mutation genotype and alcohol intake in relation to prevalence of self-reported alcohol sensitivity symptoms from the skin, lower and upper airways (nondrinkers excluded). Test for interaction between FLG genotype and alcohol consumption: $p = 0.014$. Error bars represent 95% CIs using the binomial distribution.

rier function in the gastrointestinal tract. In support of this idea, it has been found that FLG is expressed in the oral epithelium [11, 34]. However, FLG is not expressed in the esophageal mucosa [11].

Milk allergy was the most commonly reported food allergy, and this raised the concern that some cases of intolerance to the milk sugar lactose may have been misclassified as milk allergy. Therefore, we investigated whether the association between FLG mutations and self-reported allergy to milk was dependent of the -13910 C/T polymorphism upstream of the lactase-producing gene, the main genetic cause of PLI in Europe [30]. The C allele causing PLI represents the wild type, but the mutational T allele has spread in northern Europe as the ability to metabolize lactose and drink cow's milk beyond childhood may have represented a nutritional advantage during periods of limited food supply. Carriers of the C/C genotype drink, on average, less milk due to intolerance symptoms on exposure to lactose. Interestingly, we found a significant interaction between FLG mutations and PLI in relation to self-reported milk allergy. Since the ingestion of food allergens probably promotes immunological tolerance, a lower ingestion of milk due to genetically determined intolerance to lactose could therefore possibly increase the risk of IgE-mediated allergy to milk. One way or another, this interesting

gene-by-gene interaction deserves further investigations in other populations.

We found that FLG mutations were significantly associated with an increased risk of alcohol sensitivity. Moreover, there was a significant interaction between FLG mutations and alcohol consumption in relation to alcohol sensitivity. Of note is that the association between FLG genotype and alcohol sensitivity was independent of the presence of asthma, hay fever and atopic dermatitis. The possible biological mechanisms underlying this association are unknown. It could be of interest that FLG gene transcripts have been found in stomach tissue, which may suggest that FLG is present in the mucosa of the stomach [13]. Although the biological function of FLG in the stomach is not known, FLG mutations may interact with the well-known effects of alcohol on the stomach in terms of gastritis and increased gastrointestinal permeability. It may also be of interest that alcohol has an influence on the immune system and may impair the Th1-helper lymphocyte cell-mediated immune response. Furthermore, it is well documented that alcohol consumption increases serum total IgE and the risk of specific IgE sensitization to cross-reactive carbohydrate determinants, which are abundant in foods, while the effects of alcohol on IgE sensitization to inhalant allergens are not clear [35–41]. The relevance of these effects for the apparent effects of FLG mutations on risk of alcohol sensitivity is not known. In this study, we could not distinguish between alcohol sensitivity and histamine intolerance. The natural histamine content in wine has been proposed as the substance responsible for red wine-induced asthmatic reactions, but the results from double-blind challenge tests did not support this hypothesis; thus, Kanny et al. [42] observed an increase in plasma histamine after the intake of histamine-poor wine, but surprisingly no increase after intake of histamine-rich wine. The authors concluded that substances other than the histamine content in wine are responsible for intolerance reactions to wine. An alternative explanation for the association between FLG mutations and alcohol sensitivity could be that FLG mutation carriers drink more alcohol to suppress itching in the skin. As expected for a Caucasian population, we have previously reported that the ALDH2 487lys variant known to be strongly related to alcohol sensitivity and drinking in Asians is extremely rare in our background population [43], and other less strong genetic variants may play a role in alcohol sensitivity.

There are several limitations to this study. First, no questions on peanut allergy were included in the questionnaire, and we were therefore not able to examine the

previously reported association between FLG mutations and peanut allergy. There are limited data on the prevalence of peanut allergy in Denmark. Osterballe et al. [44] found that the prevalence of self-reported and challenge-confirmed peanut allergy in 22-year-olds was 5.3 and 0.6%, respectively.

Second, self-reported food allergy is considered to be less reliable than, for example, measurements of IgE sensitization to food allergens or food challenge. However, in a subsample of the population, we confirmed that FLG mutation carrier status was associated with IgE against milk, wheat and codfish, although this was only statistically significant for sIgE positivity to milk. Furthermore, we have no reason to believe that misclassification of food allergy would be differentially related to FLG genotype. It may thus be speculated that the nondifferential misclassification of food allergy has attenuated the true associations of food allergy with FLG genotype.

In conclusion, the observed strong association between FLG mutations and self-reported food allergy may provide new insights into the pathogenesis of food allergy and the well-established link between food allergy and atopic dermatitis. More studies using objective measures of food allergies are warranted.

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