



Filaggrin Gene Mutations with Special Reference to Atopic Dermatitis

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Abstract

Purpose of review Mutations in the filaggrin gene can cause absent or reduced filaggrin protein, leading to impaired keratinization and skin barrier defect, which produce characteristic phenotypes. In this short review, we report current evidence on the topic with special reference to atopic dermatitis, suggest future directions, and discuss therapeutic implications.

Recent findings Numerous candidate gene association studies, genome-wide association studies, studies on copy number variations, and, most recently, sequencing studies have confirmed the robust association of mutations in the filaggrin gene with atopic dermatitis, and have also linked these mutations with several other disorders.

Summary Filaggrin gene defects remain the strongest identified genetic risk factors for atopic dermatitis. Taken in conjunction with other genes found to be associated with this condition, genetic screening and identification of individuals at risk for atopic dermatitis could lead to personalized therapy. Manipulation of genetic regulatory elements to increase the amount of filaggrin protein in deficient individuals is an attractive treatment option for the future.

Introduction

The filaggrin gene (*FLG*) is one of the 70 genes forming the Epidermal differentiation complex (EDC), a 2 MB region located on human chromosome 1q21. These genes are responsible for the maturation of keratinocytes, which

are the chief cells in human epidermis. Keratinocytes play a crucial role in maintaining an intact skin barrier. In 2006, two landmark studies from Dr. WH McLean's group demonstrated the association of loss-of-function (LOF) mutations in *FLG* with ichthyosis vulgaris [1••], a condition characterized by impaired keratinization resulting from either homozygous or compound heterozygous mutations in the gene that has a dominant/semi-dominant mode of inheritance, and atopic dermatitis (AD) [2••], a complex disorder characterized by an impaired skin barrier in the presence of an allergic milieu. Since then, numerous

studies have replicated the *FLG*-AD association from the original study, and several other variants in *FLG* have been shown to be associated with AD. Indeed, the strength of association between *FLG* and AD is exceptionally high for a complex disease, with the overall odds ratio determined to be 3.12 (95% CI 2.57, 3.79) for the combined genotype that included the two most common genetic variants in a large meta-analysis [3•]. Also, *FLG* remains the most replicated gene in AD [4••]. In this short review, we provide a summary of the role of *FLG* variants in AD as well as in some other diseases.

Short biology of *FLG*

FLG consists of 2 introns and 3 exons, the third exon being the largest and the chief coding element. *FLG* codes for the precursor protein profilaggrin, which undergoes dephosphorylation and cleavage to form 10–12 copies of the active filaggrin protein [5]. The *FLG* mutations that have been found associated with AD are located in the third exon of the gene and are loss-of-function (LOF) mutations, mostly reported as nonsense (stop-gain) ones. A LOF mutation can be present in only one copy of the gene (heterozygote) or in both copies (homozygote), or two different LOF mutations can be present in the same person, producing a compound heterozygote. All LOF mutations in *FLG* have precisely the same effect, i.e., reduction in the amount of filaggrin protein produced. This reduction is dependent on the number of LOF mutations the individual carries, with a heterozygote having a less severe phenotype than a homozygote or a compound heterozygote and likely, the location of the LOF mutation on the third exon, as well as on the number of copies of the tandem repeats coding filaggrin protein (discussed later).

FLG in AD

Epidemiology of AD

AD is a chronic, relapsing disorder characterized by red, itchy skin rashes that predominantly affects children, though the presence of an adult form is now well-established. The prevalence of childhood AD ranges from 15 to 25% worldwide, and current evidence suggests that it is increasing. AD is a disease that carries significant patient-level, social, and economic burden [6], and in addition, can be the first step in “atopic march,” a triad of diseases starting with AD followed by the later development of asthma and allergic rhinitis [7, 8]. AD is a highly heritable disease. In a systematic review, the concordance rate for AD in monozygotic twins was found to be up to 86% in monozygotic twins, compared to up to 41% in dizygotic ones, with the overall concordance ratio for monozygotic versus dizygotic twins being close to 3, highlighting the importance of genetic factors over environmental ones [9]. In the same review, the heritability (the amount of phenotypic variation in a trait that can be explained by genetic variation) of AD was determined to be about 75%.

Hypotheses about the pathogenesis of AD

AD was long viewed as a primary immunological disorder, with the skin manifestations presumed to be secondary to an internal dysfunction of the immune system (the inside-outside view). However, according to the outside-inside hypothesis [10•], the primary defect and the driving force in AD lie in an impaired skin barrier, which allows greater allergen penetration. This leads to downstream allergen sensitization and initiation of an allergic reaction in the epidermis, presumably in the context of an already existing TH2-cytokine milieu of allergic inflammation, resulting in the full-blown picture of AD. The most robust evidence in support of the outside-inside mechanism comes from the now well-established link between LOF mutations in *FLG* and AD.

Candidate gene, GWAS, and sequencing studies

The first study to examine the association between *FLG* LOF mutations and AD was performed by Palmer et al. in 2006 [2••]. In this candidate gene association study, the investigators demonstrated strong association of two LOF mutations in *FLG*, R501X, and 2282del4, with AD, and asthma in the context of AD. The prevalence of these mutations was about 9% in this study cohort of European descent. In the wake of this discovery, numerous candidate gene association studies of AD employing different study designs were performed across different racial and ethnic groups around the world, which have examined, in addition to the two LOF mutations previously mentioned, several other variants in *FLG* (reviewed in [4••]). Candidate gene association studies start with an a priori hypothesis based on biological and functional significance of the gene. These studies were successful in firmly establishing the role of *FLG* LOF mutations in the development of AD. Genome-wide association studies (GWAS), which are agnostic in nature, examine the whole genome and have the ability to identify genes and variants that have not been previously associated with a disease. Several genome-wide association studies (GWAS) that include meta-analyses, performed across populations as diverse as European, Asian, and African were able to replicate the association of *FLG* with AD (reviewed in [11••]). More than 500 stop-gain mutations in *FLG* are now described in the genome aggregation database (gnomAD), a project that assembles and summarizes exome and genome sequencing data from chiefly diseased focused consortia ([12], gnomad.broadinstitute.org). It is noteworthy that the prevalence of *FLG* LOF variants is race specific; e.g., the most frequent variants found in Asian populations, African populations, and white populations are not the same. The most common *FLG* variants found in the white population of Northern European origin include c.2282del4, p.R501X, p.R2447X, c.3702delG, and p.Ser3247X; whereas c.441delA, c.1249insG, c.3321delA, p.Ser2554X, c.7945delA, p.Gln2147X, and p.Glu2422X are common in Asians [13]. Moreover, *FLG* LOF variants are less common in people of African descent but still present [14•]. The most common variants in African-Americans are p.S3316X, p.R826X, p.R501X, and p.S761fs [15]. Some earlier reports appeared to indicate that *FLG* LOF did not occur in those of African ancestry, but these studies may have suffered from lack

of appropriate technique [16]. At least one study has also noted that in individuals of African ancestry, another gene, *FLG2*, that has properties similar to that of *FLG*, is associated with the phenotype of persistent AD [17]. Lately, whole-exome sequencing studies, which are able to identify rare variants in the protein coding regions of the gene, have reported uncommon variants in *FLG* to be associated with susceptibility to AD in Bangladeshi families [18] and susceptibility and persistence of AD in African-American children [14•]. Several thousand *FLG* variants have been identified to date; for an up to date listing of variants by race, the readers are referred to gnomAD ([12], gnomad.broadinstitute.org).

CNV studies

As stated earlier, *FLG* exhibits intragenic copy number variation (CNV). Alleles can have between 10 and 12 monomers, with the number of copies proportional to gene dosage. The first study to examine the association of *FLG* CNV with AD was performed by Brown et al. in 2012 on an Irish population [19•]. In their control group, 51.5% had the 11-repeat allele, followed by 33.9% with the 10-repeat allele and 14.6% with the 12-repeat allele. After excluding carriers of LOF *FLG* mutations, the controls were found to have significantly greater number of *FLG* repeats as compared to cases of AD. Two subsequent studies, the first on a Russian-Tartar population [13] and the second on an Ethiopian population [20], however, were unable to demonstrate a difference in CNV prevalence among AD cases and controls; while a third study that used African-American children with AD showed a significant association between CNV dosage and severe AD [21]. Admixture in African-Americans has been suggested as a reason behind these contrasting results [20], and further studies are needed to elucidate the role of *FLG* CNVs in AD patients of African origin.

Gene-gene interactions, gene-environment interactions, and epigenetics

AD is a disease of multifactorial etiology, with the phenotype an outcome of interaction among multiple genes belonging to the skin barrier pathway and the immune pathway, and environmental factors. The interplay between genes involved in maintaining skin barrier function and immune regulation has not been extensively explored. While *FLG* remains the strongest genetic risk factor for AD, with up to half of the AD patients carrying a *FLG* mutation [2••], it is to be noted that AD can develop independently of *FLG* mutations and not all individuals who carry these mutations develop AD. This highlights the role of other genes, identified and not yet identified, as well as environmental influences, and the complex interaction between these risk factors, in the pathogenesis of AD.

Wan et al. studied the potential interaction between *FLG* and *TSLP*, a gene involved in maturation of antigen-presenting cells [22] but did not find any evidence of *TSLP* modifying the effect of *FLG* on the age of AD onset. However, using a British cohort, Ziyab et al. was able to demonstrate epistasis between *FLG* mutations and variants in another immune pathway gene, *IL-4R*, in the development of a related phenotype, allergic sensitization [23].

Important environmental exposures that could influence the development of AD include exposure to environmental allergens from pets and house dust early in life. Interaction of *FLG* mutations with cat but not dog ownership in the development of AD has been demonstrated in Danish and British cohorts by Bisgaard et al. [24]. In this study, the investigators found no effect of dust mites on AD development. Using a Dutch cohort, Schuttelaar et al. confirmed the findings from the previous study that early-life exposure to cat augmented the effect of *FLG* 2282del4 mutation in the development of AD [25]. In a study conducted in Japanese children, Ait et al. found that exposure to house dust did not increase the risk of AD in children who carried *FLG* LOF mutations; however, this study suffered from several major methodological limitations as described by the authors [26]. A report from a cohort of Swedish hairdressers, who had increased dermal level of chemical exposure, could not find an association between *FLG* LOF variants & CNV10 carrier status and AD; the lack of association was attributed to the increased prevalence of AD among hairdressers as an occupational group [27]. *FLG* LOF mutations have also been shown to modify the effect of smoking on asthma risk in a Danish population; however, the effect of these mutations on asthma risk was independent of AD status in this study [28].

Epigenetic modifications are heritable changes in gene expression that do not involve a change in the DNA sequence of an organism, and can occur due to environmental influence. The high heritability observed in AD (~75%) is only partially explained by the hitherto identified genetic variants, including *FLG*, through candidate gene studies and GWAS. While unidentified genetic variants, especially rare ones, are presumed to exist and are expected to be increasingly identified through exome and genome sequencing studies, epigenetic mechanisms could be an alternate and feasible explanation for the missing heritability. Using a British cohort of 18-year old girls, Ziyab et al. demonstrated that differential DNA methylation of *FLG*, an epigenetic mechanism, interacted with *FLG* variants to confer a 5.5 times higher risk of AD [29•]. A parent-of-origin effect was demonstrated in AD by Esperaza-Gordilla et al.; in their study of European families, children of mothers with *FLG* LOF mutations and allergic sensitization were at 1.5 times higher risk of developing AD; this risk was independent of the child's own *FLG* status. This study demonstrated for the first time that maternal environment during pregnancy could directly influence the development of a complex disease in the offspring in humans [30].

Evolutionary advantage

From the evolutionary point of view, it has been hypothesized that the skin barrier defect resulting from *FLG* LOF mutations allows greater skin penetration of UV-B, leading to an increased synthesis of Vitamin D3. This confers a survival advantage to populations living in higher latitude areas where sunlight is scarce [31, 32•]. Thyssen et al., using a meta-analysis to explore the association between the global prevalence of *FLG* LOF mutations and latitude, demonstrated that the prevalence of *FLG* LOF mutations

increases with latitude, the highest prevalence being among Northern Europeans [32•]. A later study and review using enhanced sequencing methods [33] challenged this hypothesis; however, the original proponents of the hypothesis considered their argument invalid because of the inherent inadequacies in the currently available genome databases [34]. Future studies are expected to shed light on this topic.

FLG in other disorders

FLG LOF mutations have also been investigated as causative factors in several other disorders, including allergic and dermatological ones. The link with other allergic/atopic disorders (asthma and allergic rhinitis) derives its biological plausibility from the fact that AD is the first step in the “atopic march,” where the future development of asthma and allergic rhinitis in an individual is preceded by the development of AD. Variants in *FLG* have been reported to be associated with asthma in the context of AD [2••, 3•, 35], atopic asthma independent of AD [36], allergic rhinitis [35, 37], and food allergy [38, 39]. Apart from their well-known association with ichthyosis vulgaris [1••], other skin disorders that have been found to be related to *FLG* variants include urticaria and non-AD rash [40], actinic keratosis [41], hand eczema [42], and squamous cell carcinoma [43]. However, they were not found to be associated with psoriasis [44] except in Chinese populations [45, 46], oral lichenoid lesions and oral lichen planus [47], malignant melanoma [48], and basal cell carcinoma [49].

Husomoyen et al. could not find any association between *FLG* LOF mutations and the risk of type 2 Diabetes or cardiovascular disease [50]. Another study found that these mutations could modify the association between biomarkers of exposure to endocrine-disrupting chemicals and testicular function in a cohort of young men; the suggested mechanism was an increased absorption of these chemicals through a defective skin barrier [51]. An association between a SNP in *FLG* and EBV-associated gastric carcinoma has also been reported; portal of entry of micro-organisms could be a defective skin barrier [52]. A study was also able to detect an association between *FLG* LOF mutations and ischemic stroke; however, the biological plausibility behind the association could not be explained [53]. Association of *FLG* mutations with Epstein-Barr virus-associated nasopharyngeal carcinoma and gastric carcinoma has also been reported in a study [54].

Difficulty in sequencing *FLG*

Due to its structure, *FLG* is a difficult gene to sequence. Previously, Margolis et al. assessed the validity and reliability of genotyping null mutations in the gene using two different techniques, the more conventional TaqMan and the high throughput beadchip technologies. While the same method gave identical results when repeated, statistically significant differences were observed between the techniques. Based on their results, the authors concluded that the

beadchip technology was not the ideal approach for genotyping *FLG* null mutations [55]. Recently, a multiplexed targeted resequencing approach has been described by Wong et al. that has high throughput and the potential to better identify unidentified CNVs compared to the exome sequencing method [56]. It has also recently been shown that earlier informatic programs that were used to align and genotype sequence data were inadequate for properly identifying *FLG* LOF variants. An improved program became available around 2016 [16].

Future directions

FLG LOF variation is the most common and most reproduced genetic defect associated with AD, with disease severity varying by the type of mutation. Several other gene variants have also been associated with AD, and incorporating these with knowledge of *FLG* LOF variation will facilitate better identification of susceptible individuals likely to develop AD, and thereby, personalize therapy. Due to reasons that are currently unknown, *FLG* is more prone to variation than most other EDC genes; also, the prevalence of *FLG* LOF mutations varies significantly by race. Unraveling these puzzles could lead to better understanding of the evolutionary reasons behind *FLG* LOF variants, and likely, AD.

Therapeutic implications

Despite *FLG* LOF mutations being the most common gene defects associated with AD, therapies that directly address the diminished production of filaggrin protein due to gene variation are not currently available. More severe AD is associated with *FLG* LOF variations, but severity varies by type of LOF; for example, white children with the variant p.R501X are least likely to respond to topical therapy. As noted, *FLG* LOF variations diminish the amount of filaggrin produced, and by manipulating genetic regulatory elements, it may be possible to increase the amount of filaggrin produced in individuals with these variations. However, it is to be noted that *FLG* LOF variants are not the sole reason why filaggrin production is diminished in AD; patients without these mutations but with severe skin inflammation can also have reduced filaggrin levels and consequently, impaired barrier function [57•, 58, 59]. It is likely that therapies that are effective in AD-associated inflammation indirectly improve filaggrin production. There is evidence that the type 2 cytokines, Interleukin (IL)-4 and IL-13, which are crucial to AD pathogenesis, suppress *FLG* expression; the drug dupilumab, which is a dual inhibitor of IL-4 and IL-13 signaling by its antagonistic effect on their shared receptor, IL-4 Receptor Alpha, has been shown to increase *FLG* expression in the lesional skin of AD patients in a recent study [60]. Aryl hydrocarbon receptor, which is a ligand-dependent transcription factor and a major regulator of *FLG* expression in normal skin, has also been shown to increase *FLG* expression in normal human epidermal keratinocytes through *OVOL1*, a susceptibility gene for AD identified through GWAS studies [61].

Compliance with Ethical Standards

Conflict of Interest

Jayanta Gupta reports being a co-investigator in a grant from NIAMS. David Margolis reports grants from NIAMS, personal fees from Pfizer, personal fees from Sanofi/Regeneron, grants from Valeant, and personal fees from Leo outside the submitted work.

Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors.

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