Archives of Dermatological Research (2024) 316:606
https://doi.org/10.1007/s00403-024-03273-w

RESEARCH LETTER

Novel loss-of-function variants in filaggrin exon 3 in patients with severe atopic dermatitis

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Received: 8 May 2024 / Revised: 9 July 2024 / Accepted: 5 August 2024

Dear Editor,

The strongest genetic risk for atopic dermatitis (AD) is variants in the filaggrin gene (*FLG*). *FLG* encodes a large protein called profilaggrin, i.e. the precursor of filaggrin which plays a multifaceted role in maintaining skin barrier function by contributing to hydration via natural moisturizing factor formation, corneocyte structural integrity, pH regulation, antimicrobial defence, and lipid barrier formation [1]. *FLG* consists of 2 introns and 3 exons. The third exon is the largest and the chief coding element. The identification of causative variants is challenging, due to high sequence homology within the 10 to 12 tandem repeats [2]. The primary objective of this study is to assess the variant frequency of *FLG* in patients with severe AD vs. healthy individuals. A cross-sectional study was conducted in consecutive patients with severe AD who were visited at the Dermatology Unit of the University Hospital of Verona (Italy) and underwent peripheral blood sampling for genetic testing at the Diagnostics Unit of the MAGI'S LAB (Rovereto, Italy). Next generation sequencing (NGS) and third-generation sequencing (TGS) were performed. TGS technologies have been applied to address the constraint posed by the sequence homology within tandem repeat regions of *FLG* gene exon 3 [3]. The inclusion criteria were signed informed consent, Italian ancestry (*defined by genealogical records in parents and grandparents)*, clinical diagnosis of AD on the basis of Hanifin and Rajka criteria, severe AD (defined as Eczema Area and Severity Index [EASI] > 20) [4]. Exclusion criteria were late disease onset (i.e.>12 years). The inclusion criteria for healthy controls were absence of any current or past diseases and/or medical conditions (i.e. no history of AD, asthma, food allergy, rhino-conjunctivitis or ichthyosis). A total of 184 individuals were included, of those 111 with severe AD and 73 healthy controls. The characteristics of the individuals are shown in Table 1. The variant frequency of *FLG* was 16 out of 111 (14.4%) in AD vs. 1 out of 73 (1.3%) in healthy controls, (Fisher's test, $p < 0.01$). Of the 16 patients with *FLG* variants, four (25%) shared the same specific variant, c.1501 C > T, p(Arg501*), that has been already reported in literature. In the other patients, the following variants were found to be novel (not found in the literature or recorded in the ClinVar database) or unpublished (recorded in the ClinVar database): c.8720G > A, p.(Trp2907*), c.7688G > A, p.(Trp2563*), c.10570G > T, p.(Gly3524*), c.6367 C > T, p.(Gln2123*), c.1165 C > T, p.(Gln389*), c.560dup, p.(Thr188Aspfs*2) c.4271_4272del, p.(Lys1424Argfs*25), and NM_002016:c.6867_6868del, p.(Arg2289Serfs*31). These are caused by single nucleotide variants, duplications and deletions leading to protein loss of function (LOF) (Table 2). Our findings align with previous studies reporting a high prevalence of deleterious variants in *FLG* in AD patients. C.1501 C > T, p.(Arg501*) variant has been extensively studied and has been shown to be one of the most common associated with AD, especially in populations of European descent [4]. We did not find other previously reported variants such as c.2282_2285del, p.(Ser3247*), p.(Arg2447*), and c.3702del probably because of geographical selection. AD patients with *FLG* LOF variants have an increased risk of presenting with severe disease, early onset, persistence into adulthood and sensitization to aeroallergens and contact allergens [5]. In conclusion, we confirmed a higher variant frequency of pathogenetic

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Published online: 06 September 2024

Table 1 Characteristics of the individuals included in the study

	Atopic dermatitis ($N=111$)	Healthy controls ($N=73$)	P
Age, years	36.9 ± 17.2	52.5 ± 15.3	< 0.005*
Gender, male	59 (53.2)	53 (72.6)	< 0.005^
EASI score	27.5 ± 5.1	N.A.	
IgE, UI/mL	1068.6 ± 1968.2	76.6 ± 43.8	< 0.005*
prick test sensitization	67 (60.4)	0	
patch test sensitization	26 (23.4)	0	
Asthma	47 (42.3)	0	
Rhino-conjunctivitis	51 (45.9)	0	
AD family history (at least one first degree relative)	34 (30.6)	0	

Continuous and categorical variables are presented as means ± standard deviation (SD) and proportions, respectively. EASI Eczema Area and Severity Index. N.A. not applicable. * T Test, ^ Fisher's test

Table 2 Loss-of-function *FLG* variants identified using NGS and TGS with long-read sequencing in AD patients and healthy controls

Patient	*Gene*	*Zygosity*	*ClinVar Interpretation*	*Molecular consequence*	*HGVS cDNA*	*Repeat n°*
Atopic dermatitis	*FLGNM_002016*	het	NA	frameshift	*c.560dup, p.(Thr188Aspfs*2)*	*0*
Atopic dermatitis	*FLGNM_002016*	het	NA	nonsense	*c.1165 C > T, p.(Gln389*)*	*0*
Atopic dermatitis	*FLGNM_002016*	het	Pathogenic	nonsense	*c.1501 C > T, p.(Arg501*)*	*1*
Atopic dermatitis	*FLGNM_002016*	het	Pathogenic	nonsense	*c.1501 C > T, p.(Arg501*)*	*1*
Atopic dermatitis	*FLGNM_002016*	het	Pathogenic	nonsense	*c.1501 C > T, p.(Arg501*)*	*1*
Atopic dermatitis	*FLGNM_002016*	het	Pathogenic	nonsense	*c.1501 C > T, p.(Arg501*)*	*1*
Atopic dermatitis	*FLGNM_002016*	het	Likely Pathogenic	frameshift	*c.4271_4272del, p.(Lys1424Argfs*25)*	*4*
Atopic dermatitis	*FLGNM_002016*	het	NA	nonsense	*c.6367 C > T, p.(Gln2123*)*	*6*
Atopic dermatitis	*FLGNM_002016*	het	NA	frameshift	*c.6867_6868del, p.(Arg2289Serfs*31)*	*6*
Atopic dermatitis	*FLGNM_002016*	het	Pathogenic	nonsense	*c.7081 C > T, p.(Arg2361*)*	*6*
Atopic dermatitis	*FLGNM_002016*	het	Pathogenic	frameshift	*c.7467del, p.(Ser2490Leufs*110)*	*7*
Atopic dermatitis	*FLGNM_002016*	het	NA	nonsense	*c.7688G > A, p.(Trp2563*)*	*7*
Atopic dermatitis	*FLGNM_002016*	het	NA	nonsense	*c.8720G > A, p.(Trp2907*)*	*8*
Atopic dermatitis	*FLGNM_002016*	het	NA	nonsense	*c.10570G > T, p.(Gly3524*)*	*10*
Atopic dermatitis	*FLGNM_002016*	het	Pathogenic	nonsense	*c.10,969 C > T, p.(Arg3657*)*	*10*
Atopic dermatitis	*FLGNM_002016*	het	Pathogenic	nonsense	*c.10,969 C > T, p.(Arg3657*)*	*10*
Healthy control	*FLGNM_002016*	het	Pathogenic	nonsense	*c.1501 C > T, p.(Arg501*)*	*1*

AD, atopic dermatitis; het, heterozygous; HGVS cDNA, Human Genome Variation Society complementary DNA; NA, not available; TGS, third-generation sequencing; NGS, next-generation sequencing

genetic variants in *FLG* in patients with severe AD compared to healthy individuals. The utilization of innovative approaches such as TGS can facilitate the identification of novel genetic variants.

Acknowledgements This research was funded by the Provincia Autonoma di Bolzano in the framework of LP 15/2020 and by the European Union - Next Generation EU - NRRP M6C2 - Investment 2.1 Enhancement and strengthening of biomedical research in the NHS&quot.

Author contributions F.B, P.G., G.G. and M.C.M wrote the main manuscript text and all authors reviewed the manuscript.

Data availability Data is provided within the manuscript or supplementary information files.

Declarations

Competing interests The authors declare no competing interests.

References

1. Smith FJ, Irvine AD, Terron-Kwiatkowski A et al (2006) Loss-of-function mutations in the gene encoding filaggrin cause ichthyosis vulgaris. Nat Genet 38:337–342
2. Brown SJ, Kroboth K, Sandilands A et al (2012) Intragenic copy number variation within filaggrin contributes to the risk of atopic dermatitis with a dose-dependent effect. J Invest Dermatol 132:98–104
3. Mareso C, Albion E, Cozza W, Tanzi B, Cecchin S, Gisondi P et al (2023) Optimization of long-range PCR protocol to prepare filaggrin exon 3 libraries for PacBio long-read sequencing. Mol Biol Rep 50(4):3119–3127

4. Palmer CN, Irvine AD, Terron-Kwiatkowski A, Zhao Y, Liao H, Lee SP et al (2006) Common loss-of-function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis. Nat Genet 38(4):441–446
5. van den Oord RA, Sheikh A (2009) Filaggrin gene defects and risk of developing allergic sensitisation and allergic disorders: systematic review and meta-analysis. BMJ 339:b2433