Filaggrin gene polymorphisms in Indian children with atopic dermatitis: A cross-sectional multicentre study

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Abstract

Background: Filaggrin (*FLG*) gene encoding the protein filaggrin plays an important role in barrier function of the skin and its alteration is a predisposing factor for atopic dermatitis. *FLG* gene variants result in absent or decreased filaggrin protein. Worldwide, the prevalence of *FLG* variants ranges from 14 to 56%. *FLG* null variants are distinct in each population.

Objectives: To study the *FLG* gene polymorphisms in Indian children and attempt a genotype-phenotype correlation in atopic dermatitis.

Methods: This was a cross-sectional, multicentre study conducted on 75 Indian children. Demographic details, clinical features and identified *FLG* null variants were recorded. We performed a whole gene sequencing of the entire *FLG* coding region using next-generation sequencing technology.

Results: The prevalence of *FLG* null variants was 34.7%. A total of 20 different *FLG* loss of function variants in 26 children were documented. Sixteen (80%) variants were novel and four (20%) were previously reported in Asian and European populations. We found a statistically significant association between *FLG* variants with early age of onset of atopic dermatitis ($P = 0.016$) and elevated serum IgE levels ($P = 0.051$). There was no significant difference between atopic dermatitis phenotypes in children having one variant as compared to children harbouring two or more null variants.

Limitations: Small sample size.

Conclusion: Our study reports a unique set of *FLG* variants different from Asian and European populations, with these variants being significantly associated with an early age of onset of atopic dermatitis and elevated serum IgE levels.

Key words: Atopic dermatitis, early age, filaggrin polymorphisms, next-generation sequencing, null variants, serum IgE

Plain Language Summary

Atopic dermatitis is a chronic, itchy, relapsing inflammatory skin disorder seen more commonly in children. Filaggrin is a protein which plays an important role in maintaining the normal barrier function of the skin. Defects in the filaggrin gene can cause reduced or absent production of filaggrin, contributing to the development of atopic dermatitis. This study was conducted on 75 children at two different hospitals in India, to identify the filaggrin gene variants and assess their role in atopic dermatitis. There were 20 different filaggrin gene variants identified in 26 children in our study. Among them, 16 filaggrin gene variants were unique to our study population while four had been reported previously in Asian and European populations. These filaggrin gene variants in our study were significantly associated with an early age of onset of the atopic dermatitis and increased serum levels of the IgE class of antibodies.

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Introduction

Atopic dermatitis is a chronic, progressive, relapsing inflammatory skin disorder characterised by varying degrees of pruritus and inflammation seen in 20% of children and 1–3% of adults.[1] The prevalence of atopic dermatitis in India is around 0.5–7.2 per cent.[2] Atopic dermatitis is the result of a complex interplay of several environmental, dietary, behavioural and immunological factors in genetically predisposed individuals.[2] Filaggrin (filament aggregating protein, *FLG*) gene is an important component of skin barrier function and plays an important role in epidermal differentiation. Epidermal barrier and *FLG* null variants are thought to play a key role in the pathogenesis of atopic dermatitis, as well as in allergic rhinitis and asthma.[3] Null variation is a change in the gene that results in non-transcription of mRNA or translation of protein or both. Null variants can be due to frameshifts or nonsense substitutions. *FLG* gene studies have been carried out in many geographical populations, which include European and Asian populations (Japanese, Chinese, Korean, Taiwan and Singaporean). Patients with *FLG* variants have been reported to have more persistent and severe disease, a higher incidence of herpes virus infections, association with ichthyosis vulgaris, palmar hyperlinearity, keratosis pilaris, asthma and a greater risk of multiple allergies than patients with atopic dermatitis without *FLG* variants.[4] *FLG* gene variants may predict early onset, severe disease, worse prognosis, persistence of the disease in adulthood, allergen sensitisation and development of IgE-mediated food allergies.[5]

Several cohort studies have revealed that only 14–56% of patients harbour *FLG* gene null variants as a predisposing factor.[6,7] The profilaggrin/*FLG* gene resides on chromosome 1q21.3 within the epidermal differentiation complex and consists of three exons. Exon 3 is extremely large (>12 kb) and sequencing is challenging as it encodes most of the profilaggrin polypeptide with almost complete homologous 10, 11 and 12 repeats.[5] Null variants in exon 3 result in complete loss of the expressed protein. Every race is likely to have a unique set of *FLG* variants which could predict the severity of the disease.[4,8] Most of the studies have analysed the more common loss of function variants, which could have resulted in under-reporting of other significant loss of function variants seen in different ethnic populations. Recent technology like next-generation sequencing can analyse the entire coding region of *FLG* to identify unreported loss of function variants.[3]

A recent Indian study has shown a prevalence of variant R501X to be 2.2% in children concomitantly associated with asthma and atopic dermatitis.[9] Handa *et al*. have reported a prevalence of 33.7% of *FLG* variants (S2889X, 2282del4, R501X and Q2417X) associated with hand eczema from the Indian population.[10] *FLG* variants could predict the outcome of atopic dermatitis which would help clinicians to counsel parents about the likely severity of atopic dermatitis. We aimed to study the *FLG* gene polymorphisms in Indian children with atopic dermatitis and do a genotype-phenotype correlation.

Materials and methods

Study population

This was a, cross-sectional, multicentre study of atopic dermatitis cases over 2 years, from April 2018 through April 2020. It was conducted at two centres in India, the Department of Pediatric Dermatology, Indira Gandhi Institute of Child Health, Bengaluru, Karnataka and the Institute of Child Health, Kolkata, West Bengal. We calculated the required sample size to be 69, based on the prevalence of *FLG* gene mutations in atopic dermatitis of 31.4 per cent.[11,12] There has been no previous study in the Indian population available for comparison.

Children less than 18 years of age diagnosed with atopic dermatitis, with parents and all four grandparents of Indian ethnicity, presenting to the outpatient department of the two hospitals were enrolled. Children with mixed ethnicities were excluded. The diagnosis of atopic dermatitis was based on the criteria by Hanifin and Rajka.[13] Demographic details were recorded on a predesigned proforma. Children were classified into three age groups based on the onset of atopic dermatitis: infantile (birth to 1 year), early childhood (1–8 years) and late childhood (8–18 years). Disease severity was assessed using the SCORing Atopic Dermatitis (SCORAD) index and classified as mild (<25), moderate (25–40) and severe (>40).[14] Associated diseases like allergic rhinitis and asthma were diagnosed based on the questionnaire and previous diagnoses by a paediatrician. Written informed assent from the children and consent from the parents/guardians were obtained for clinical and molecular analysis. Institutional ethical committee clearances were taken from both the centres where the study was conducted (IEC/9/2018-19, IEC/183/2019).

Molecular analysis

Genomic DNA was extracted from peripheral blood ($n = 75$) using the genomic DNA extraction kit (Qiagen). DNA samples were amplified by polymerase chain reaction to different amplicon sizes to cover the coding regions (~13.26 kb) of the *FLG* gene (three exons) using the specific primers. The amplicons were sheared (ME220, Covaris), pooled and libraries prepared (NEBNext kit-E7370L, NEB) as per the manufacturer's protocol. The libraries were sequenced (HiSeq X, Illumina) to an average sequencing depth of ≥500x. A minimum of 90% of the sequenced bases were ≥Q30 value. The variants were called using Genome Analysis Toolkit (GATK) best practices pipeline annotated using a framework for the identification of variants in the sample using Sentieon (v201808.01). The sequences obtained were aligned to the human reference genome (GRCh37/hg19) using Sentieon aligner and analysed using Sentieon for removing duplicates, recalibration and

realignment of indels. Gene annotation of the variants was performed using a variant effect predictor program against the Ensembl release 91 human gene model.[15] Clinically relevant variants were annotated using published variants in literature and a set of diseases databases—ClinVar, OMIM, GWAS, HGMD (v2018.3) and SwissVar. Common variants were filtered based on allele frequency in 1000 Genome Phase 3, ExAC (v1.0), gnomAD (v2.1), EVS, dbSNP (v151), 1000 Japanese Genome and our internal Indian population database and *in silico* prediction tools [PolyPhen 2, Sorting Intolerant From Tolerant (SIFT), Mutation Taster2 and likelihood ratio test (LRT)]. The variants were analysed using Varminer (in-house variant interpretation tool) and prioritised. Around 100 age and sex matched controls were selected among the samples received for clinical exome sequencing for some other condition.

Statistical analysis

Descriptive statistics for quantitative values were expressed as means (± standard deviation) in accordance with the data distribution. Frequencies and percentages were used to describe categorical variables. Fisher's exact test was used to compare parameters between atopic dermatitis with *FLG* variants and atopic dermatitis without *FLG* variants, as well as atopic dermatitis-associated phenotypes that included gender, family history of atopy, allergic disease association, pityriasis alba, palmar hyperlinearity, xerosis, cheilitis, SCORAD; and a Chi-square test for comparing the age of onset. *P* value was obtained from Student's unpaired *t*-test for age at presentation. The *P* value for the significance of IgE was obtained from Mann–Whitney *U* test. The association between the occurrence of mutation and various phenotypes and clinical features was obtained by Spearman's rank correlation (rho) and expressed along with confidence intervals. A similar Spearman's rank correlation was done to interpret the association between the number of variants (single or more) and various phenotypic expressions and clinical parameters. MedCalc version 10.2 (MedCalc Software, 2011, Mariakerke, Belgium) was used for statistical analysis. A 'P' value of less than 0.05 ($P < 0.05$) was considered statistically significant.

Results

Clinical characteristics of study children

We recruited 80 unrelated children diagnosed with atopic dermatitis in the study. Five children failed the DNA quality control (insufficient DNA sample), hence we analysed 75 children [Figure 1]. Among these 75 children, 50 were boys and 25 were girls aged 4 months to 18 years (mean age, 5.2 ± 3.9 years). The clinical characteristics of children with and without *FLG* variants are presented in Table 1. The mean age of onset of the disease was 2.7 years (10 days to 12 years). Around 93.4% of children presented with atopic dermatitis in the early age group (birth to 8 years). Seventy children (93.3%) had moderate to severe disease in our study. In the present cohort, palmar hyperlinearity was seen in 42.7% and

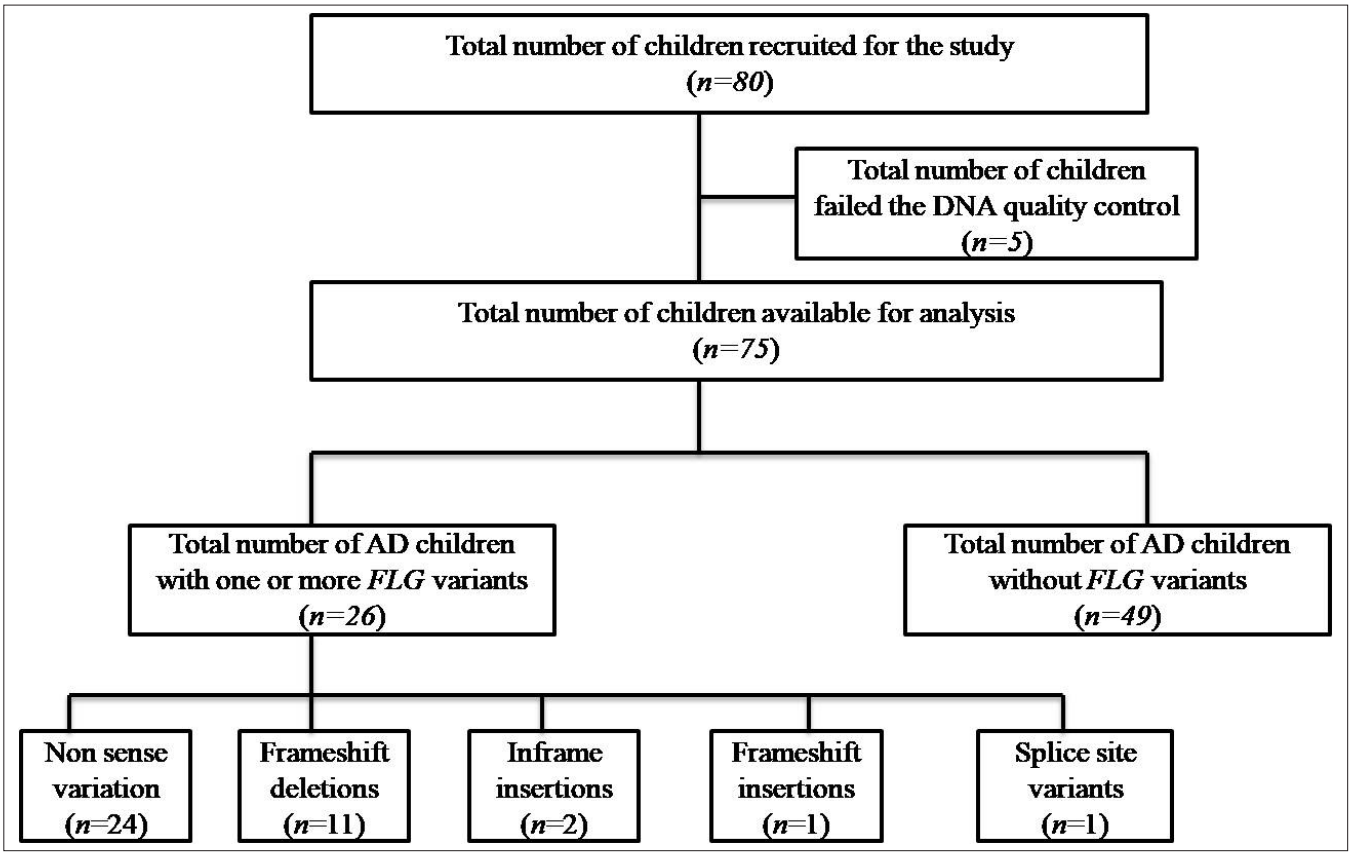

Figure 1: Flow chart of the study

Table 1: Clinical characteristics of Indian children with and without *FLG* variants

Clinical features	AD with *FLG* variants (*n* = 26)	AD without *FLG* variants (*n* = 49)	Total (*n* = 75)	*P*
Age at enrolment (years)				
Mean ± SD	4.57 ± 3.97	5.61 ± 3.88	5.25 ± 3.92	0.277
Range	0.4–13	0.4–18	0.4–18	
Median (IQR)	3.2 (1.8, 6)	5 (2.45, 8.13)	4 (2, 8)	
Gender				
Boys	16 (61.5%)	34 (69.4%)	50 (66.7%)	0.608
Girls	10 (38.4%)	15 (30.6%)	25 (33.3%)	
Age of onset				
Infantile (birth–<1 year)	17 (65.4%)	18 (36.7%)	35 (46.7%)	0.041
Early childhood (1–8 years)	7 (26.9%)	28 (57.1%)	35 (46.7%)	
Late childhood (8–18 years)	2 (7.7%)	3 (6.1%)	5 (6.7%)	
Family history	10 (38.5%)	22 (44.9%)	32 (42.7%)	0.632
Allergic disease association (allergic rhinitis and/or asthma)	10 (38.5%)	24 (49.0%)	34 (45.3%)	0.468
SCORAD				
Mild (<15)	1 (3.8%)	4 (8.2%)	5 (6.7%)	0.653
Moderate (15–40)	16 (61.5%)	35 (71.4%)	51 (68%)	0.440
Severe (>40)	9 (34.6%)	10 (24.4%)	19 (25.3%)	0.264
Pityriasis alba	6 (23.1%)	14 (28.6%)	20 (26.7%)	0.785
Palmar hyperlinearity	12 (46.2%)	20 (40.8%)	32 (42.7%)	0.807
Xerosis	21 (80.8%)	36 (73.5%)	57 (76%)	0.577
Cheilitis	3 (11.5%)	12 (24.5%)	15 (20%)	0.234

P value <0.05 was considered significant. SD, Standard deviation; IQR, Interquartile range; AD, Atopic dermatitis; *FLG*, Filaggrin gene; SCORAD: SCORing Atopic Dermatitis

xerosis in 76% of children. Serum IgE levels could be done in only 55 children due to logistical reasons.

Molecular results

This study documented 26 children having one or more *FLG* null variants. The prevalence of *FLG* loss of function mutation was 34.7%. The data on *FLG* loss of function variants in our study are summarised in Table 2. There were 20 different *FLG* loss of function variants in our study. Of these 24 (61%) were nonsense mutations, 11(28%) frameshift deletions, 1(3%) frameshift insertions, 1(3%) splice loss of function variants and 2(5%) inframe insertions. Among the 20 *FLG* loss of function, 16 (80%) were unique to our study, not reported in any database or literature [Table 3]. All these novel variants were not detected in 100 people who did not have *FLG* variants. Four variants (20%) in ten patients in our study were previously reported null variants (shown in bold in Table 2: p.Arg2447Ter, p.Arg501Ter, c.2282del4 and p.Arg2037Ter). Seven variants were detected in more than one individual of whom four were novel (p. Ser3640Ter, p.Ser2344Ter, p.Arg992SerfsTer31 and p.Trp1064Ter) and three (p.Ser761CysfsTer36, p.Arg2447Ter and p.Arg501Ter) were previously reported null variants. Two variants, nonsense (p. Ser3640Ter) and frameshift (p.Ser761CysfsTer36) were detected in five patients each and another nonsense variant (p.Ser2344Ter) in four patients. Three (11.5%) children had homozygous null variants (p.Ser761cysfsTer36, p.Arg99SerfsTer31 and p.Arg501Ter) and 23 (88.5%) children had heterozygous null variants. Among the 13 children with more than one variant, 11 (42.3%) had two null variants and two (7.7%) had three null variants.

Association of *FLG* null variants with atopic dermatitis

In our study *FLG* variants were significantly associated with early age of onset ($P = 0.016$) and elevated serum IgE levels which just reached statistical significance ($P = 0.051$) [Table 4]. About 65.4% of children with *FLG* variants presented with atopic dermatitis in the infantile age group. Though 96% of atopic dermatitis children with *FLG* variants had moderate to severe atopic dermatitis, it was not a statistically significant finding ($P = 0.154$). A negative correlation between pityriasis alba and *FLG* variants was found [Table 4]. Clinical characteristics of 13 (50%) children who had two or more *FLG* null variants are described in Table 5. No significant associations were observed between the *FLG* genotype with two or more null variants and severity or other atopic dermatitis-associated phenotypes, except the early age of onset [Table 6].

Discussion

There are more than 500 stop-gain variants in *FLG* described in the genome aggregation database.[16] The most common *FLG* variants shared among Japanese, Koreans,

Table 2: Loss of function variants of the *FLG* gene in Indian children in the present study

Patient ID	Transcript ID	VarClass	cDNA change	AA change	Zygosity	dbSNP ID	Variant
237870	NM_002016.2	NONSENSE	c.1339C>T	p.Gln447Ter	Heterozygous	rs763028697	chr1:152286023G>A
237870	NM_002016.2	INTRONIC-SS-ACR	c.139-1G>A	NA	Heterozygous	–	chr1:152287224C>T
288486	**NM_002016.2**	**NONSENSE**	**c.6109C>T**	**p.Arg2037Ter**	**Heterozygous**	**rs200002200**	**chr1:152281253G>A**
303109	**NM_002016.2**	**NONSENSE**	**c.7339C>T**	**p.Arg2447Ter**	**Heterozygous**	**rs138726443**	**chr1:152280023G>A**
196655	**NM_002016.2**	**NONSENSE**	**c.7339C>T**	**p.Arg2447Ter**	**Heterozygous**	**rs138726443**	**chr1:152280023G>A**
327946	**NM_002016.2**	**NONSENSE**	**c.1501C>T**	**p.Arg501Ter**	**Heterozygous**	**rs61816761**	**chr1:152285861G>A**
261722	**NM_002016.2**	**NONSENSE**	**c.1501C>T**	**p.Arg501Ter**	**Homozygous**	**rs61816761**	**chr1:152285861G>A**
237872	**NM_002016.2**	**NONSENSE**	**c.1501C>T**	**p.Arg501Ter**	**Heterozygous**	**rs61816761**	**chr1:152285861G>A**
303026	NM_002016.2	NONSENSE	c.1714C>T	p.Arg572Ter	Heterozygous	rs200601767	chr1:152285648G>A
303026	NM_002016.2	FRAMESHIFT-DEL	c.2976_2977del	p.Arg992SerfsTer31	Heterozygous	rs776968118	chr1:152284384GCT>G
261725	NM_002016.2	FRAMESHIFT-DEL	c.2976_2977del	p.Arg992SerfsTer31	Heterozygous	rs776968118	chr1:152284384GCT>G
254989	NM_002016.2	FRAMESHIFT-DEL	c.2976_2977del	p.Arg992SerfsTer31	Homozygous	rs776968118	chr1:152284384GCT>G
327941	NM_002016.2	FRAMESHIFT-DEL	c.5014del	p.Gln1672ArgfsTer34	Heterozygous	rs771090956	chr1:152282347TG>T
261717	NM_002016.2	NONSENSE	c.6940C>T	p.Gln2314Ter	Heterozygous	rs762689918	chr1:152280422G>A
237856	NM_002016.2	NONSENSE	c.9315C>G	p.Tyr3105Ter	Heterozygous	rs200815866	chr1:152278047G>C
227344	NM_002016.2	FRAMESHIFT-INS	c.4808_4812dup	p.Glu1605ThrfsTer103	Heterozygous	rs775716153	chr1:152282549C>CTGAGT
303034	NM_002016.2	FRAMESHIFT-DEL	c.6067_6082del	p.Gly2023HisfsTer67	Heterozygous	–	chr1:152281279GAAGCTTGTCCATGCCC>G
227335	NM_002016.2	NONSENSE	c.7777G>T	p.Gly2593Ter	Heterozygous	rs756353784	chr1:152279585C>A
196661	NM_002016.2	NONSENSE	c.1279G>T	p.Gly427Ter	Heterozygous	rs544327834	chr1:152286083C>A
237894	NM_002016.2	FRAMESHIFT-DEL	c.8590_8591del	p.His2864CysfsTer5	Heterozygous	rs1407703398	chr1:152278770ATG>A
343834	NM_002016.2	NONSENSE	c.7031C>G	p.Ser2344Ter	Heterozygous	rs372754256	chr1:152280331G>C
343833	NM_002016.2	NONSENSE	c.7031C>G	p.Ser2344Ter	Heterozygous	rs372754256	chr1:152280331G>C
256713	NM_002016.2	NONSENSE	c.7031C>G	p.Ser2344Ter	Heterozygous	rs372754256	chr1:152280331G>C
254986	NM_002016.2	NONSENSE	c.7031C>G	p.Ser2344Ter	Heterozygous	rs372754256	chr1:152280331G>C
343834	NM_002016.2	NONSENSE	c.10919C>G	p.Ser3640Ter	Heterozygous	rs745827275	chr1:152276443G>C
343833	NM_002016.2	NONSENSE	c.10919C>G	p.Ser3640Ter	Heterozygous	rs745827275	chr1:152276443G>C
327948	NM_002016.2	NONSENSE	c.10919C>G	p.Ser3640Ter	Heterozygous	rs745827275	chr1:152276443G>C
256713	NM_002016.2	NONSENSE	c.10919C>G	p.Ser3640Ter	Heterozygous	rs745827275	chr1:152276443G>C
254986	NM_002016.2	NONSENSE	c.10919C>G	p.Ser3640Ter	Heterozygous	rs745827275	chr1:152276443G>C
343830	**NM_002016.2**	**FRAMESHIFT-DEL**	**c.2282_2285del**	**p.Ser761CysfsTer36**	**Heterozygous**	**rs558269137**	**chr1:152285076CACTG>C**
288486	**NM_002016.2**	**FRAMESHIFT-DEL**	**c.2282_2285del**	**p.Ser761CysfsTer36**	**Heterozygous**	**rs558269137**	**chr1:152285076CACTG>C**
261725	**NM_002016.2**	**FRAMESHIFT-DEL**	**c.2282_2285del**	**p.Ser761CysfsTer36**	**Heterozygous**	**rs558269137**	**chr1:152285076CACTG>C**
254979	**NM_002016.2**	**FRAMESHIFT-DEL**	**c.2282_2285del**	**p.Ser761CysfsTer36**	**Homozygous**	**rs558269137**	**chr1:152285076CACTG>C**
227339	**NM_002016.2**	**FRAMESHIFT-DEL**	**c.2282_2285del**	**p.Ser761CysfsTer36**	**Heterozygous**	**rs558269137**	**chr1:152285076CACTG>C**
343834	NM_002016.2	NONSENSE	c.3191G>A	p.Trp1064Ter	Heterozygous	rs546421276	chr1:152284171C>T
343833	NM_002016.2	NONSENSE	c.3191G>A	p.Trp1064Ter	Heterozygous	rs546421276	chr1:152284171C>T
227344	NM_002016.2	NONSENSE	c.7434C>G	p.Tyr2478Ter	Heterozygous	rs144157090	chr1:152279928G>C

Previously reported null variants are highlighted in bold. VarClass, variant classifier; cDNA, complementary DNA; AA, amino acid; dbSNP ID, single nucleotide polymorphism database; FRAMESHIFT-DEL, frameshift deletion; FRAMESHIFT-INS, frameshift insertion

Table 3: Novel *FLG* loss of function variants detected in the present study

Patient ID	VarClass	cDNA change	AA change	Allele frequency	gnomAD	dbSNP ID
237870	INTRONIC-SS-ACR	c.139-1G>A	NA	0.006666667	NA	–
303026	NONSENSE	c.1714C>T	p.Arg572Ter	0.006666667	0.00009746	rs200601767
327941	FRAMESHIFT-DEL	c.5014del	p.Gln1672ArgfsTer34	0.006666667	0.00002843	rs771090956
261717	NONSENSE	c.6940C>T	p.Gln2314Ter	0.006666667	0.00001218	rs762689918
237856	NONSENSE	c.9315C>G	p.Tyr3105Ter	0.006666667	0.00001361	rs200815866
237870	NONSENSE	c.1339C>T	p.Gln447Ter	0.006666667	0.00000406	rs763028697
303034	FRAMESHIFT-DEL	c.6067_6082del	p.Gly2023HisfsTer67	0.006666667	NA	–
227335	NONSENSE	c.7777G>T	p.Gly2593Ter	0.006666667	0.00000812	rs756353784
196661	NONSENSE	c.1279G>T	p.Gly427Ter	0.006666667	0.00000812	rs544327834
343834, 343833, 327948, 256713, 254986	NONSENSE	c.10919C>G	p.Ser3640Ter	0.033333333	0.00006558	rs745827275
343834, 343833	NONSENSE	c.3191G>A	p.Trp1064Ter	0.013333333	0.00015025	rs546421276
227344	NONSENSE	c.7434C>G	p.Tyr2478Ter	0.006666667	0.00001218	rs144157090
303026, 261725, 254989	FRAMESHIFT-DEL	c.2976_2977del	p.Arg992SerfsTer31	0.026666667	0.0001868	rs776968118
227344	FRAMESHIFT-INS	c.4808_4812dup	p.Glu1605ThrfsTer103	0.006666667	0.00000812	rs775716153
237894	FRAMESHIFT-DEL	c.8590_8591del	p.His2864CysfsTer5	0.006666667	NA	rs1407703398
343834, 343833, 256713, 254986	NONSENSE	c.7031C>G	p.Ser2344Ter	0.026666667	0.0004731	rs372754256

VarClass, variant classifier; cDNA, complementary DNA; AA, amino acid; dbSNP ID, single nucleotide polymorphism database; FRAMESHIFT-DEL, frameshift deletion; FRAMESHIFT-INS, frameshift insertion; gnomAD, genome aggregation database

Table 4: Correlation between *FLG* variants and clinical features

Clinical features	Correlation coefficient (rho)	Confidence interval	*P*
Age of onset of atopic dermatitis (months)	−0.277	−0.475 to −0.0539	0.016
Family history of atopy	0.0619	−0.167 to 0.285	0.598
SCORAD severity	0.166	−0.063 to 0.379	0.154
Allergic disease association (allergic rhinitis and/or asthma)	−0.0997	−0.319 to 0.130	0.395
Serum IgE levels (IU/mL)	0.264	−0.001 to 0.495	0.051
Pityriasis alba	−0.276	−0.473 to −0.0524	0.0165
Palmar hyperlinearity	0.0514	−0.178 to 0.275	0.6617
Xerosis	0.0813	−0.148 to 0.303	0.4878
Cheilitis	−0.154	−0.368 to 0.0755	0.1869

Taiwanese, Chinese, Singaporeans and Asians are R501X and c.3321delA. In the European population, the most prevalent mutations are R501X and c.2282del4. The three most common *FLG* variants R501X, c.2282del4 and E2422X are shared in both European and Asian populations.[8] Meng *et al.* found c.3321delA to be associated with xerosis, ichthyosis vulgaris, palmar hyperlinearity, keratosis pilaris, white dermographism and disease severity but not associated with early age of onset.[17] Previous studies found that *FLG* loss of function variants were not commonly seen in persons of African ancestry, which could be due to inappropriate genotyping techniques. Recent studies with massively parallel sequencing have shown uncommon *FLG* variants to be associated with persistent atopic dermatitis in African ancestry as compared to European ancestry. However, the frequency of *FLG* loss of function mutations in African ancestry is lower. Copy number variation has been associated as a risk factor for atopic dermatitis and this has been documented in African-American pediatric patients with moderate to severe atopic dermatitis.[18]

Table 5: Genotype and phenotype of children with atopic dermatitis carrying more than one *FLG* null variant

Patient ID	Variant	Variant_1	Variant_2	Variant_3	Age of onset	Family history	Allergic disease association	SCORAD	Other features
227344	Two heterozygous variants	p.Glu1605ThrfsTer103	p.Tyr2478Ter	NA	10 days	Wheezing	Nil	Moderate	Xerosis
237856	Two heterozygous variants	p.Gln2659_Gln2660insLeu	p.Tyr3105Ter	NA	1 month	Nil	Nil	Moderate	Xerosis/hyperlinearity
237870	Two heterozygous variants	c.139-1G>A	p.Gln447Ter	NA	3 months	Nil	Nil	Severe	Xerosis/hyperlinearity/cheilitis
254979	Homozygous	p.Ser761CysfsTer36	p.Ser761CysfsTer36	NA	6 months	Nil	Nil	Moderate	Xerosis/hyperlinearity
254986	Two heterozygous variants	p.Ser2344Ter	p.Ser3640Ter	NA	6 months	Nil	Nil	Moderate	Xerosis/hyperlinearity
254989	Homozygous	p.Arg992SerfsTer31	p.Arg992SerfsTer31	NA	6 months	Nil	Allergic rhinitis	Moderate	Xerosis/hyperlinearity/periorbital darkening
256713	Two heterozygous variants	p.Ser2344Ter	p.Ser3640Ter	NA	5 months	Atopic dermatitis	Nil	Moderate	Xerosis/hyperlinearity
261722	Homozygous	p.Arg501Ter	p.Arg501Ter	NA	12 years	asthma	Allergic rhinitis	Moderate	Nil
261725	Two heterozygous variants	p.Arg992SerfsTer31	p.Ser761CysfsTer36	NA	3 months	Nil	Allergic rhinitis	Severe	Xerosis
288486	Two heterozygous variants	p.Arg2037Ter	p.Ser761CysfsTer36	NA	4 months	Wheezing	Nil	Moderate	Xerosis/hyperlinearity
303026	Two heterozygous variants	p.Arg572Ter	p.Arg992SerfsTer31	NA	10 days	Asthma	Allergic rhinitis	Severe	Xerosis
343833	Three heterozygous variants	p.Ser2344Ter	p.Ser3640Ter	p.Trp1064Ter	3 months	Nil	Nil	Moderate	Keratosis pilaris
343834	Three heterozygous variants	p.Ser2344Ter	p.Ser3640Ter	p.Trp1064Ter	3 months	Nil	Nil	Mild	Nil

NA, Not applicable

Table 6: Correlation between children having more than one *FLG* null variants and clinical features

Clinical features	Correlation coefficient (rho)	Confidence interval	*P*
Age of onset of atopic dermatitis (months)	−0.614	−0.809 to −0.297	0.0008
Family history of atopy	0.00	–	1.00
SCORAD severity	−0.277	−0.600 to 0.124	0.1711
Allergic disease association (allergic rhinitis and/or asthma)	−0.203	−0.547 to 0.200	0.3203
Serum IgE levels (IU/mL)	−0.208	−0.596 to 0.258	0.3782
Pityriasis alba	−0.548	−0.771 to −0.204	0.0038
Palmar hyperlinearity	0.154	−0.248 to 0.511	0.4517
Xerosis	−0.0976	−0.467 to 0.301	0.6353
Cheilitis	−0.120	−0.485 to 0.280	0.5580

About 20 *FLG* loss of function variants in 26 children (6 frameshift, 13 nonsense and 1 splice site variant) were seen in our study.

Among the documented *FLG* loss of function variants in our study, 20% of the variants were previously reported in European and Asian populations and 80% were unique variants.[16,17,19,20] India is home to more than 2000 ethnic populations, whose genetic origins are complex. The present Indian population is genetically heterogeneous with an admixture of ancestral north Indians comprising the majority and having roots in the Middle East, Central Asia and Europe and ancestral south Indians that have no relation to any population outside India.[21] Further complexity is added by the relatively recent immigration of populations such as Siddis, Muslims and Jews.[22] Ours being a two-centre study,

had both these sets of populations (East Indians and South Indians) and thus reported variants of Asia and European as well as a high number of unique variants. Chauhan *et al.* studied the prevalence of R501X mutation in Indian children with allergic diseases. Their study was a case-control study study enrolling 90 children with allergic diseases (asthma, rhinitis and atopic dermatitis). The mutant R501X was seen in 3.3% of children with asthma and 2.2% in children with asthma concomitantly with eczema. The limitation of their study was that screening of only one *FLG* null variant was done.[9]

Several studies have shown the strong association of *FLG* null mutation with different atopic dermatitis phenotypes.[16] *FLG* null variants in our study were significantly associated with early age of onset (infantile) and elevated serum IgE levels, which was concordant with several earlier studies.[23,24,25] *FLG* variants have a significant effect on the age of onset of atopic dermatitis and can lead to early onset (less than 1 year) and persistence of the disease into adulthood.[23,24] Although previous studies have shown a significant association between the *FLG* null variants with moderate to severe atopic dermatitis (odds ratio 2.03–13.4), our study did not show a statistically significant correlation.[4,11,26,27] *FLG* mutation is a strong genetic predisposing factor for atopic dermatitis, but other factors like unidentified candidate genes, copy number variations and epigenetic factors may also play an important role in the pathogenesis of atopic dermatitis, which could explain the non-correlation of the severity of atopic dermatitis with *FLG* variants in our study, as well as the fact that SCORAD is variable at different points of time.[16] Interestingly Park *et al.* in a study on Korean patients with atopic dermatitis did not observe any significant association between *FLG* null variants and clinical features of atopic dermatitis.[18]

Ota *et al.* studied the effect of *FLG* loss of function mutation on the severity of skin lesions and skin barrier function in the Japanese population. Eight (14.5%) patients had a loss of function mutations in the *FLG* gene in their study. Stratum corneum was collected from three different sites (extremities, neck and trunk) using the tape-stripping method. The amount of *FLG* protein and total amino acid in the stratum corneum was measured in both mutation carriers and non-carriers. *FLG* abnormalities had little effect on the severity of dermatitis, *FLG* protein and total amino acid content in the stratum corneum in the lesional skin. Their study suggested that the activation of Th2 dominant inflammatory cells along with *FLG* abnormalities plays a role in suppressing the production of *FLG* in lesional skin.[28]

Previous studies have shown *FLG* mutation to be associated with elevated levels of IgE in Japanese, Koreans, Singaporeans, Chinese and European populations.[4,29] In our study too, serum IgE levels were significantly associated with *FLG* null variants. We were unable to find any association between *FLG* variants with allergic disease association, family history, palmar hyperlinearity, xerosis, cheilitis and keratosis pilaris and this was similar to other studies.[4,7,26]

We found no significant associations in severity or clinical manifestations of atopic dermatitis between children carrying one single variant and children with two or more variants. This could again be explained by the multifactorial nature of the disease. Though *FLG* mutation is not an independent risk factor for the development of different atopic dermatitis phenotypes, studies on *FLG* variants may help identify some infants at risk of developing atopic dermatitis, guiding early interventions to delay the onset and decrease the severity of atopic dermatitis.

Limitations

The main limitation of our study is its relatively small sample size. As a result, there could have been false negative or positive results due to low statistical power. The small sample size in our study also did not permit a statistical correlation between specific null variants and atopic dermatitis phenotypes. Larger population-based studies are required to substantiate the associations between *FLG* mutations and atopic dermatitis phenotypes in the Indian population. We could not look for copy number variation in the present study due to logistic reasons.

Conclusion

Our study documented a high prevalence of *FLG* null variants in Indian children. The *FLG* variants identified in our study included ones unique to this population and also those that overlapped with Asian and European populations. The presence of more than one *FLG* mutations in an individual did not correlate significantly with the severity of AD. We found a significant association of *FLG* variants with an early age of onset and elevated serum IgE levels.

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Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent.

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Conflicts of interest

There are no conflicts of interest

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