Original Article

Genotype Study of Filaggrin Gene Loss-of-Function Mutations in Central India Population with Atopic Dermatitis and Ichthyosis Vulgaris

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Abstract

Background: A genotype study of filaggrin gene loss-of-function mutations in central India can provide valuable insights into the prevalence and association of these mutations with atopic dermatitis (AD) and ichthyosis vulgaris (IV) in the region. The *FLG* R501X and 2282del4 are both genetic variants in the human gene called filaggrin gene (*FLG*), which encodes a protein that plays an important role in the formation and maintenance of the skin barrier. In this study, we determined the *FLG* R501X and 2282del4 variants association with both AD and IV in Central Indian populations. **Materials and Methods:** This case–control study was conducted in the Departments of Dermatology and Molecular and Virology Research and Diagnostic Laboratory at Sri Aurobindo Medical College and Post Graduate Institute, Indore (Madhya Pradesh). The study was approved by the Clinical Research and Ethics Committee. A total of 180 patients aged between 3 months - 60 years who attended the skin outpatient department between March-2021 to June-2022 were recruited in this study. Among them, 60 patients were in AD-group, 60 patients in IV-group, and 60 patients were in the healthy control group. Polymerase chain reaction followed by restriction fragment length polymorphism (PCR-RFLP) was used in genotyping for *FLG* mutations (R501X and 2282del4). **Results:** The most common *FLG* mutations were R501X (31.6% and 23.3%) and 2282del4 (18.3% and 13.3%) in AD and IV patients with heterozygous (AT) genotype, respectively. The combined mutation (*FLG* R501X and 2282del4) association was 10% and 5% in the AD and IV groups with heterozygous (AT) genotype, respectively, and in all the patients of control group with wild genotype (AA). There were no significant (P = 0.09) associations found with 2282del14 genotype. **Conclusion:** The R501X mutation in the gene encoding filaggrin is one of the robust genetic associations of AD and IV. The 2282del4 polymorphism was marginally less as compared to R501X.

Keywords: *Atopic dermatitis, filaggrin, ichthyosis vulgaris, R501X and 2282del4*

Introduction

A genotype study of filaggrin gene loss-of-function mutations in Central India can provide valuable insights into the prevalence and association of these mutations with atopic dermatitis (AD) and ichthyosis vulgaris (IV) in the region. Filaggrin (*FLG*) is an epidermal protein that plays an important role in the terminal differentiation of epidermis and formation of the stratum corneum. A mutation in the gene encoding *FLG* has been identified as the cause of IV and is a major predisposing factor for AD.[1] *FLG* gene is encoded within the epidermal differentiation complex on chromosome 1q21.[2,3] The *FLG* loss-of-function is of great significance for barrier impairment in AD and IV. The most common mutations in European studies include R501X and 2282del4,[3] and the carrier frequencies differ substantially from 1% to 4% in an Italian population and 4% to 56% in an Irish population.[4] One of the first association studies[4] revealed that *FLG* null mutations predispose individuals to AD, and numerous studies later confirmed this observation, with odds ratios (ORs) ranging from approximately 2 to 7.[5-7] These mutations are significantly associated with AD.[8] There are marked differences in the prevalence of *FLG* mutations detected in northern versus southern European populations. The prevalence of *FLG* mutations ranges from 25% to 50% in certain northern European populations of AD patients, while in southern European populations, *FLG* mutations are

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uncommon or even absent, with rates ranging from 0.5% to 4%.[3,4,9-12] Very few studies have assessed the prevalence and association of *FLG* mutations and specifically no study has been done in a cohort of AD and IV patients and a corresponding control group in the Central Indian population. In this study, we aimed to characterize the relationship between the two most prevalent mutations (*FLG* R501X and 2282del4) in individuals with AD/IV and a group of healthy controls.

Materials and Methods

Study population

The cross-sectional study was conducted in the Departments of Dermatology and Molecular and Virology Research and Diagnostic Laboratory at Sri Aurobindo Medical College and Post Graduate Institute, Indore (Madhya Pradesh). The study was approved by the Clinical Research and Ethics Committee. A total of 180 patients aged between 3 months - 60 years who attended the skin outpatient department between March-2021 to June-2022 were recruited in this study. Among them, 60 patients were in AD-group, 60 patients in IV-group, and 60 patients were in the healthy control group.

Study designs

The AD was diagnosed in patients with UK working party diagnostic criteria. Patients were diagnosed to have IV based on clinical history and examination and histopathological evaluation. Patients not having features of AD and IV were selected randomly by a second observer in the control group (e.g., family history of atopic or allergic illnesses, signs of atopic eczema, asthma, or hay fever, food allergies, pollen allergies, or other environmental allergies are some examples of exclusion criteria). In this study, all patients aged between 3 months-60 years were enrolled. Written informed consent was taken from all the patients. Severity was scored using the scoring atopic dermatitis (SCORAD) index,[13,14] and patients were classified as mild (SCORAD <15), moderate (SCORAD 15-40), or severe (SCORAD >40).[15,16]

Genotyping analysis

Genomic DNA was obtained from peripheral blood using the QIAamp DNA Blood Mini Kit (QIAGEN). Genotyping for the *FLG* mutations R501X and 2282del4 was performed by polymerase chain reaction (PCR) fragment amplification followed by conventional PCR and electrophoresis. The primers used were designed based on the findings of previous studies and modified to our PCR optimization protocol.[15]

Genotyping of R501X polymorphism

Genomic DNA was amplified by using Applied Biosystems 2720 Thermal Cycler PCR (Forward Primer, Reverse primer, TaqBuffer (Tris without MgCl2), MgCl2, dNTP Mix, Taqpolymerase). Initial denaturation at 94°C for 5 min followed by 35 cycles of denaturing at 94°C for 40s, annealing at 60°C for 60s, extension at 72°C for 90s, and a final incubation at 72°C for 7 min. The resulting amplified PCR product was run in 2% agarose gel (120 bp, 200bp) and the PCR product was digested with Hin1II (NIaIII) (Thermo Scientific) restriction enzyme and incubated at 37°C for 3 hours. After digestion, the product was run on 2.5% agarose gel [Figure 1].

Genotyping of 2282del4 polymorphism

Genomic DNA was amplified by using Applied Biosystems 2720 Thermal Cycler PCR (Forward Primer, Reverse primer, Taq Buffer (Tris without MgCl2), MgCl2, dNTP Mix, Taqpolymerase). Initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturing at 94°C for 30s, annealing at 57°C for 45s, extension at 72°C for 50s, and a final incubation at 72°C for 7 min. The resulting amplified PCR product was run in 2% agarose gel (811bp) and PCR product was digested with Adel (DraIII) (Thermo Scientific) restriction enzyme and incubated at 37°C for 3 hours. After digestion, the product was run on 2.5% agarose gel [Figure 2].

Statistical analysis

Descriptive statistics for quantitative values were expressed as the mean and standard deviation (SD), in accordance with the data distribution. Frequencies and percentages were used to describe categorical variables. Chi-squared or Fisher's exact tests were used to assess associations between *FLG* mutations and AD and IV, as well as variables associated with AD and IV including associated diseases. SCORAD index for AD was also performed. The level of statistical significance was set at $P < .05$.

Results

Clinical features

Table 1 summarizes the clinical characteristics of the sample. A total of 180 patients were included in this cohort

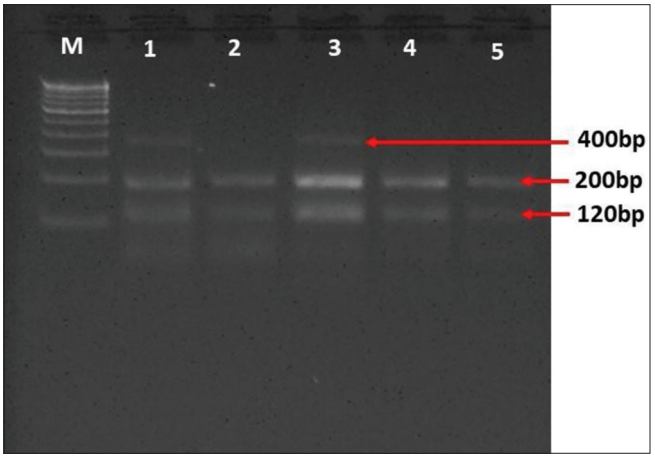

Figure 1: Showing R501X mutation. Lane M: DNA Marker/DNA Ladder (1000bp) Lane 2, 4 and 5 - Wild Allele band for R501X (200bp and 120 bp). Lane 3 - Heterozygous alleles for R501X (400 bp)

study. In each group, there were 60 patients (AD, IV, and healthy control group). The mean age ± SD (years) was 19.02 ± 12.98 in AD, 18.93 ± 8.74 in IV, and 20.8 ± 13.25 in control group. There were 31 (51.6%) males and 29 (48.1%) females in AD, 36 (60%) males and 24 (40%) females in IV, and 34 (56.6%) males and 26 (43.4%) females in control group. Based on the SCORAD index for AD group, 65% of patients were found to be mild, 25% were found to be moderate, and 10% were found to be severe.

Genotyping finding

The frequency and distribution of *FLG* mutations R501X, 2282del14, and the combined genotype for each group are shown in Table 2.

R501x mutation

The R501X mutation was seen in 31.6%, 23.3%, and 5% in heterozygous genotype (AT) and 68.3%, 76.6%, and 95% in homozygous genotype (AA) in AD, IV, and control groups, respectively. There was a significant difference found (*P* value 0.001).

2282del4mutation

The 2282del4 mutation was seen in 18.3%, 13.3%, and 3.3% in heterozygous genotype (AT) and 81.7%, 86.7%, and 96.7% in homozygous genotype (AA) in AD, IV, and control groups, respectively. There was a significant difference found (*P* value 0.33).

Combined genotype (R501X + 2282del4)

The combined mutation (R501X and 2282del14) was found to be 90%, 95%, and 100% in homozygous genotype (AA) in AD, IV, and control groups, respectively, and in heterozygous genotype (AT) it was found to be 10% in AD and 5% in IV group. There was no mutation found in control group in heterozygous genotype. There was no significant difference found (*P* value 0.086).

Association between FLG mutations based on SCORAD indexing AD group

The association between *FLG* mutations based on SCORAD index in AD group is shown in Table 3. There was a significant (*P* = 0.001) association found with R501X and combined mutation (R501X + 2282del14) in AD group. There were no significant (*P* = 0.97) association found with 2282de14 genotype.

Discussion

Filaggrin is the main component of the keratohyalin granules in the stratum granulosum. It is present in the granular layers in the form of pro-fillagrin and on further flattening and terminal differentiation of cells, this profillagrin breaks into multiple identical copies of filaggrin monomers. In the stratum corneum, filaggrin breaks down further to form natural moisturizing factors (NMF). NMF has several physiological roles in a normal skin like UV protection, maintaining pH of stratum corneum, and water retention.[17] It has been proven in previous studies that filaggrin-deficient skin is more prone to have increased penetration of chemicals and allergens and it may lead to higher incidence of

Table 1: Clinical characteristics of the study

	AD Group (*n*=60)	IV Group (*n*=60)	Control Group (*n*=60)
Mean Age±SD (years)	19.02±12.98	18.93±8.74	20.8±13.25
Gender			
Male, *n* (%)	31 (51.6%)	36 (60%)	34 (56.6%)
Female, *n* (%)	29 (48.1%)	24 (40%)	26 (43.4%)
Severity (SCORAD Index)			
Mild, *n* (%)	39 (65%)	-	-
Moderate, *n* (%)	15 (25%)	-	-
Severe, *n* (%)	6 (10%)	-	-

SD: Standard Deviation; *n*: Number: AD: Atopic Dermatitis; IV: Ichthyosis Vulgaris

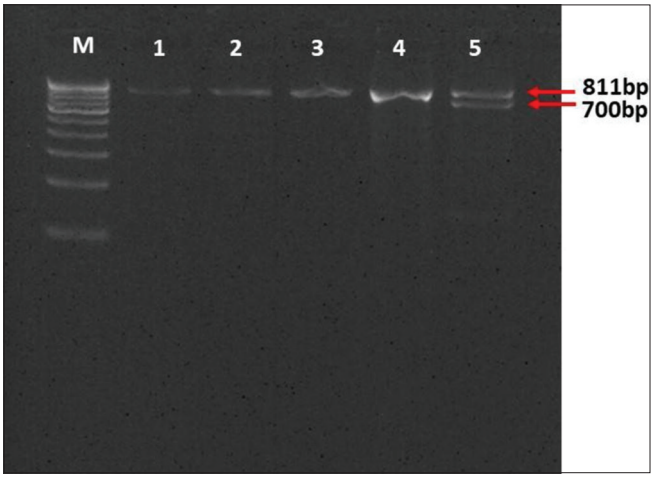

Figure 2: Showing 2282del4 mutation. Lane M: DNA Marker/DNA Ladder (1000bp). Lane 1, 2, 3 and 4 - Wild Allele Band for 2282del4 (811bp). Lane 5 – Heterozygous Alleles for 2282del4 (811bp and 700bp)

Table 2: Association of *FLG* mutations between three groups

	AD Group (*n*=60)		IV Group (*n*=60)		Control Group (*n*=60)		*P*
	AT	AA	AT	AA	AT	AA	
R501X	19 (31.6%)	41 (68.3%)	14 (23.3%)	46 (76.6%)	3 (5%)	57 (95%)	0.001
2282del4	11 (18.3%)	49 (81.7%)	8 (13.3%)	52 (86.7%)	2 (3.3%)	58 (96.7%)	0.033
R501X + 2282del4 (Combined genotype)	6 (10%)	54 (90%)	3 (5%)	57 (95%)	0	60 (100%)	0.086

AT: Heterozygous Genotype; AA: Homozygous wild-type Genotype; AD: Atopic Dermatitis; IV: Ichthyosis Vulgaris

Table 3: Genotypic polymorphism in patients with according to severity in AD Group

	Mild	Moderate	Severe	*P*
R501X				
AT	3 (5.0%)	11 (18.3%)	3 (5%)	0.001
AA	36 (60%)	4 (6.6%)	3 (5%)	
2282del14				
AT	4 (6.6%)	3 (5%)	2 (3.3%)	0.277
AA	35 (58.3%)	12 (20%)	4 (6.6%)	
R501X + 2282del14				
AT	0 (0.0%)	2 (3.3%)	3 (5%)	0.001
AA	39 (65%)	13 (21.6%)	3 (5%)	

hand eczemas, irritant contact dermatitis, and other dermatitis.[18] This research study was done to determine the association of the most common *FLG* loss-of-function mutations (R501X, 2282del4) in an Indian population of AD and IV patients. The frequency of *FLG* loss of function mutations in R501X and 2282del4 was 31.6% and 18.3% in AD group, 23.3% and 13.3% in IV group, and 5% and 3.3% in healthy control group, respectively, in heterozygous genotype (AT). Similar results reported in previous studies by Barker (2007).[5] The prevalence of the *FLG* loss-of-function mutations R501X and 2282del4 was (25.80% and 20.85%, respectively) in AD group, and R501X and 2282del4 was (2% and 1% respectively) in control group. However, the frequency of *FLG* loss of function mutations in R501X and 2282del4 in IV group were similar reposted by previous study.[19] Our finding showed the combined mutation (R501X and 2282del14) in heterozygous genotype (AT) was 10% in AD and 5% in IV group. The results were consistent with the previous studies by Sandilands *et al*.,[20] Palmar *et al*.,[21] Hubiche *et al*.,[22] Samdani *et al*.,[23] and Trzeciak *et al*[24]. Only 18.3% patient of AD in this study had heterozygous (AT) genotype of 2282del4 polymorphism, whereas only 13.3% patients of IV showed heterozygous (AT) genotypes.There was a significant difference seen ($P = 0.33$). Such low frequency of mutation was also reported in studies by Gao *et al*.,[25] and Giardina *et al*.[26] Both AD and IV are chronic diseases and the development of drugs targeting *FLG* replacement in stratum corneum should be focused more in future. Direct *FLG* replacements by topical application are being researched, but delivering such a large monomer into the skin is a challenge. However, in a concept study by Stout *et al*.,[27] topical application of engineered *FLG* showed some success in cell culture, skin equivalents, and mouse models. Topically applied functional *FLG* monomers are able to penetrate epidermal tissue, be internalized into the appropriate cell type, and be processed to a size similar to wild-type functional barrier peptides to restore necessary barrier function.[27] For further improvement in such therapeutical application of *FLG* in patients of AD and IV and other dermatosis, large-scale similar studies are required from different geographical populations.

Conclusion

This research deepens our understanding of *FLG* mutations in AD and IV patients in India, supporting the previous view of the involvement of R501X mutations in the pathogenesis of AD and IV, similar to what is reported in European and Pakistani populations. However, this study indicated that the occurrence of the 2282del4 mutation was comparatively low, suggesting the possibility of other *FLG* gene polymorphisms or other genes potentially involved in patients with atopic dermatitis and ichthyosis vulgaris. Association between *FLG* mutations and severity of diseases in AD group was significantly found with R501X mutation and also with combined R501X and 2282del4 genotype mutation. To confirm our finding, there is a need for large sample volume and multicenter study in the Indian population.

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Conflicts of interest

There are no conflicts of interest.

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