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Exploring Genetic Variants in Epidermal Differentiation Complex Genes in Severe Atopic Dermatitis: A Case Series

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Objective: The complex interplay between genetic and external factors contributes to the multifactorial nature of atopic dermatitis (AD). The study aimed to use next-generation sequencing (NGS) to identify and describe genetic alterations and polymorphisms in the epidermal differentiation complex (EDC) in two children with severe atopic dermatitis. **Methods:** A case-series study was conducted involving two children with severe atopic dermatitis, selected from a group of 103 based on questionnaire data, clinical manifestations (SCORAD index, skin moisture, trans-epidermal water loss), and laboratory tests (total IgE levels). Whole-exome sequencing (WES) was performed to analyze their genomic DNA. **Results:** Among the two participants, gene variants related to skin conditions, allergies, autoimmune disorders, and neurometabolic disorders were identified. Both participants exhibited variants in FLG, HRNR, and SPRR1B genes located in the Epidermal differentiation complex. Among these genetic variants, classifications such as "VUS/Weak Pathogenic" and "Likely Pathogenic" were observed, and synonymous variants were found alongside missense. A significant finding was the identification of rare alleles not documented in allele frequency databases. **Conclusion:** Identifying various alleles highlighted those multiple gene variants, acting together, may contribute to the development of the disease, warranting further investigation.

Keywords: Epidermis, High-throughput nucleotide sequencing, Filaggrin proteins, Genes, Dermatitis, atopic

Introduction

Approximately 15–25% of the population have atopic dermatitis (AD), and environmental factors and genetic predispositions interact, resulting in this complex, heterogeneous skin

condition.[1] The underlying pathophysiology of AD involves both immune system dysregulation and impaired skin barrier function.[2,3]The skin, particularly the epidermis, is the body's primary defense against external factors, with the outermost layer playing a crucial role in maintaining barrier integrity.[4] Epidermal barrier integrity relies on a well-regulated process of epidermal differentiation governed by a complex set of genes. Epidermal differentiation complex (EDC) comprises around 60 genes, which can be divided into five types: small proline-rich proteins, late cornified envelope proteins, S100 family, and S100 fused type protein (SFTP) family. A mutation causes skin barrier dysfunction, which leads to allergens penetrating the skin and causing inflammation and hypersensitivity.[3] The FLG gene encodes filaggrin, which is essential for bundling keratin filaments and maintaining the structural integrity of the epidermis. Thus, mutations in the FLG gene have been shown to contribute to the early onset of atopic dermatitis.[5] Given that this condition commonly manifests at a young age in our population, there is a high likelihood of polymorphisms and mutations in FLG and other relevant genes.

The main goal of performing a WES analysis in this recent study was to consider all genes within the EDC to contribute to the skin barrier function and pathogenesis of AD. One of the most significant achievements in modern biotechnology is next-generation sequencing (NGS), including methods such as targeted NGS and whole exome sequencing (WES). Sequencing tools are essential in developing personalized medicine, not just for research purposes. These sequencing methods are the key to discovering the causes of genetic disorders, complex diseases, and conditions.[6] Recent studies exploring modifying genes responsible for genetic mutations have garnered considerable attention from medical researchers, underscoring the potential for transformative advancements in genetic medicine.[7,8] In his case series, we explore genetic mutations and polymorphisms associated with the Epidermal differentiation complex in two Mongolian children with atopic dermatitis and investigate potential alterations in skin barrier function, including trans-epidermal water loss and skin pH, about observed gene expression changes.

Materials and Methods

Study Design and Participants.

This study was conducted as a case series between April and May 2023. A total of 103 children aged 0–18 years diagnosed with AD based on established clinical criteria were initially enrolled. Children were excluded if they had other significant dermatological conditions, were currently using systemic immunosuppressive therapy, or had used moisturizers in the past 24 hours or topical treatments in the past 7 days. Two participants were selected as the most severe cases for further analysis to explore genetic variants associated with AD.

Clinical evaluation

Criteria based on the SCORAD (Scoring Atopic Dermatitis) index, skin moisture levels, trans-epidermal water loss (TEWL), and total immunoglobulin E (IgE) levels were used to select the two most severe cases. Clinical evaluations involved an initial assessment of detailed medical history using the International Study of Asthma and Allergies in Childhood (ISAAC) tool and confirmation of diagnosis through a clinical examination using the Hanifin Rajka criteria.[9-10] The severity of the disease was assessed using the SCORAD index, which combines three main components: extent of lesions, intensity of lesions, and subjective symptoms of AD into a single score. The index classifies severity into three categories: mild (0–25 points), moderate (25–50 points), and severe (>50 points).[11]

Skin physiological parameters were evaluated according to the European Group on Efficacy Measurement and Evaluation (EEMCO) guidelines using the Multi Skin Test MC750 instrument (Courage+Khazaka electronic GmbH, Köln, Germany), with probes placed perpendicular to the skin surface.[12-14] Measurements of skin moisture, TEWL, and pH were performed at nine sites: cheek, forehead, abdomen, interscapular region, back of the thigh, groin, volar forearm, dorsal forearm, and antecubital fossa. The instruments used included the Corneometer® CM 825 for skin moisture, Tewameter® TM Hex for TEWL, and Skin-pH-Meter® PH 905 for skin pH. Serum total IgE levels were measured using the ELISA method with AccuBind ELISA Microwells (Monobind LLC, Lake Forest, CA, USA).

Genetic analysis

Based on questionnaires, clinical evaluation, and laboratory tests, two children with the most significant findings were selected for WES and assigned the codes ID4 and ID102. Genomic DNA was extracted from samples of selected participants using

a DNA blood mini kit from Qiagen, Germany. This process was done at the Clinical Molecular Diagnostic Center of MNUMS, the first step of WES analysis.

The following steps of WES analysis by NGS method were performed at Macrogen Inc., South Korea; the library preparation was performed using SureSelect Human All Exon v7 capture kit (Agilent LLC., USA) for all protein-encoding data. The prepared library was sequenced using NovaSeq 6000 (Illumina LLC., USA). Primary nucleotide sequence data were processed on BAM, BAM. BAI and VCF files, along with specialized bioinformatics software, and the analysis results, along with annotations, were obtained from the company. Data analysis methods were employed to identify mutations and polymorphisms. BAM and VCF files of primary whole exome sequence data were reanalyzed using bioinformatics platforms with Martin Luther University Halle-Wittenberg, Germany. This process included opening the BAM and VCF files with the Integrative Genomics Viewer (IGV) program to identify changes in nucleotide sequences compared to reference gene sequences. Additional resources utilized for analysis included GeneCards (https://www.genecards.org/), OMIM (https://www.omim.org/), Mutation Taster (https://www.mutationtaster.org/), PolyPhen-2 (https://genetics.bwh.harvard.edu/pph2/), and ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) to assess the probability of disease using predictive analysis across multiple platforms. Furthermore, allele frequencies detected in the population were comparatively studied using the Ensemble platform (https://grch37.ensembl.org/info/genome/variation/species/populations.html) to verify the elements recorded in the database.

Statistical Analysis

Descriptive statistics were performed using STATA 14 (Stata Corporation LLC, USA) software. Statistical processing was conducted according to the relevant criteria for each research material, and the actual probability was determined.

Ethical Statement

The study was initiated with the approval of the Ethics Committee of the Mongolian National University of Medical Sciences on May 20, 2022, through the resolution of the meeting (Decision No. 2022/3-05). Approval for sending specimens was obtained from the Ministry of Health Ethics Committee on December 21, 2023, through the meeting resolution (Decision No. 23/077). Informed consent was obtained from all participants before they were included in the study.

Results

Results for clinical characteristic

In our study, disease severity based on the SCORAD index showed that 17 participants (16.5%) had mild atopic dermatitis, 50 participants (48.5%) had moderate, and 36 participants (35%) had severe disease. All participants' mean skin moisture was 36.27±7.25%, TEWL was 9.01±3.44 g/m^2/h, and skin pH was 5.29±0.49. The total IgE level was measured at 118.8 [2.3-458].

Two participants (coded ID4 and ID102) with the highest SCORAD indexes, significant changes in skin physiological measurements, elevated total IgE levels, and disease history of AD, as noted in the family medical history questionnaire, were selected for performing whole exome sequencing (WES) (Table 1).

Table 1. The general and clinical characteristics of the study of the two selected participants

Parameters	Participants	
	ID4	ID102
Age, years	14	4
Gender	Female	Female
Age of AD onset (years)	0	1
Family medical history	2 siblings	2 siblings
Chronic and relapsing	Yes	Yes
Lesion lasts for more than 6 months	Yes	Yes
SCORAD index score (points)	65.4	66.5
Severity of the disease	Severe	Severe
Skin moisture (%)	29.37	37.11
TEWL ($g/m^2/h$)	7.22	8.22
Skin pH	6.1	5.4
Total IgE level (IU/ml)	458	406.4
Dyslipidaemia	5.01	3.59, 7.08

Whole exome sequencing (WES) results

The raw data (BAM and VCF files) of primary WES data and interpretation results were from the Macrogen NGS clinic. When comparing the nucleotide sequences identified through WES analysis with the reference genome, 404 nucleotide sequence variations were found in child ID4, and 744 were found in child ID102. In child ID4, genetic changes were more prevalent in genes regulating the immune and nervous systems. In contrast, child ID102 showed a higher prevalence of genetic changes associated with skin, allergic, and autoimmune disorders. Each nucleotide sequence variation was analyzed using the GeneCards and OMIM platforms and categorized by organ system disorders based on their direct or indirect impact on the pathogenesis of atopic dermatitis (AD). The findings are presented in a table format (Table 2, 3). Among the two most affected participants in our study, genetic variants linked to skin conditions, allergies, autoimmune disorders, and neurodegenerative disorders were identified. Participant ID4 exhibited 57 gene variants related to skin disorders, 251 related to immune regulation, 91 related to allergic disorders, 160 to neural disorders, and 106 to other categories. In comparison, participant ID102 showed 188 gene variants associated with skin disorders, 373 with immune regulation, 231 with allergic disorders, 179 with neural disorders, and 208 with other categories.

WES results on Epidermal differentiation complex

Both children exhibited multiple alterations in the FLG, HRNR, and SPRR1B genes. Missense and synonymous variants were observed among genetic variants, such as "VUS/Weak Pathogenic" and "Likely Pathogenic. " A significant finding was the identification of rare alleles not documented in allele frequency databases. We present the gene variants in EDC in Tables 2 and 3, showing the rs number, transcript, allele state, and exon information. Bioinformatics platforms for prediction analysis, including Mutation Taster, PolyPhen, and ClinVar, were used.

By selecting the FLG gene sequence from the DNA sequences identified through WES analysis of children ID4 and ID102 and comparing it to the reference genome using the IGV program, several heterozygous alleles were identified in exon 3. These include rs117440780 (NM_002016.2: c.12090G>A, p.Thr4030=), rs76413899 (NM_002016.2: c.2509G>A, p.Gly837Ser), and an unspecified Rs (NM_002016.2: c.1177G>T, p.Asp393Tyr).

Additionally, in the analysis of child ID4, the heterozygous allele rs751835907 (NM_002016.2: c.5732G>A, p.Ser1911Asn), rs77422831 (NM_002016.2: c.11213G>A). were identified, while in child ID102, heterozygous alleles rs1243659295 (NM_002016.2: c.8510C>G, p.Ala2837Gly), rs2338554 (NM_002016.2: c.6603T>C, p.Asp2201=), rs66977240 (NM_002016.2: c.6626A>G, p.His2209Arg), and rs66954353 (NM_002016.2: c.6695A>C, p.Lys2192Gln) were detected (Figure 1). The FLG gene variant rs751835907 in child ID4 was identified as likely pathogenic. However, the other changes were classified as benign based on predictive analysis programs, although they are rare alleles not recorded in allele frequency databases.

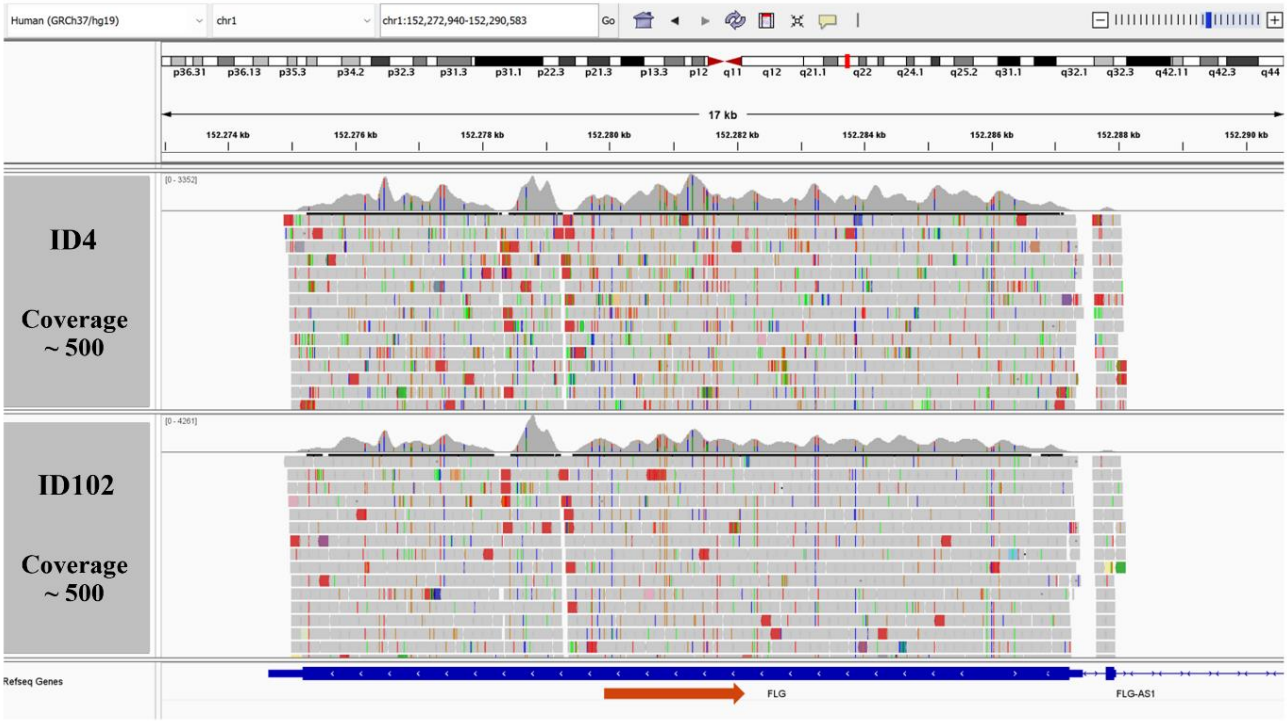

Figure 1. Comparison of the FLG gene sequences identified through WES analysis with the reference genome. Participant ID4 data is at the top, and ID102 data is at the bottom.

According to the prediction analysis, five variants in the HRNR gene and one in the SPRR1B gene were detected in participant ID4. Among these, the rs76413899 heterozygote allele in the HRNR gene is the most likely to contribute to the disease (Table 2). Twenty-six variants in the HRNR gene and three variants in the SPRR1B gene were detected in participant ID102. Among these results, the rs61814940 and rs61814941 variants in the HRNR gene were the most likely to contribute to the disease (Table 3). The heterozygote allele rs61814940 was detected in both children. All five alleles, rs61814940 (NM_001009931.3, c.5996A>T, p.Gln1999Leu), rs77376932 (NM_001009931.3, c.3160G>A, p.Glu1054Lys), rs41266134 (NM_001009931.3, c.1550A>G, p.Tyr517Cys), rs6666097 (NM_001009931.3, c.1280G>A, p.Gly427Asp), rs7545406 (NM_001009931.3, c.819C>A, p.His273Gln), in HRNR gene in participant ID4 were detected in participant ID102 (Table 2, 3).

A homozygous allele, rs3795382 (NM_003125.3, c.32C>T, p.Thr11Ile) in the SPRR1B gene is detected in both participants (Table 2, 3).

Table 2. The gene variants in the epidermal differentiation complex identified in participant ID4

Gene	Rs number	Transcript	Variant	Allele State	Exon	Sequence Ontology	Classification
FLG	rs751835907	NM_002016.2	c.5732G>A, p.Ser1911Asn	Heterozygous	3	missense variant	Likely Pathogenic
FLG	rs76413899	NM_002016.3	c.2509G>A, p.Gly837Ser	Heterozygous	3	missense_variant	Benign
FLG	rs117440780	NM_002016.2	c.12090G>A, p.Thr4030=	Heterozygous	3	missense_variant	Benign
FLG	rs unknown	NM_002016.2	c.1177G>T, p.D393Y	Heterozygous	3	missense_variant	Benign
HRNR	rs61814940	NM_001009931.3	c.5996A>T, p.Gln1999Leu	Heterozygous	3	missense_variant	VUS/Weak Pathogenic
HRNR	rs77376932	NM_001009931.4	c.3160G>A, p.Glu1054Lys	Heterozygous	3	missense_variant	Benign
HRNR	rs41266134	NM_001009931.5	c.1550A>G, p.Tyr517Cys	Heterozygous	3	missense_variant	Benign
HRNR	rs6666097	NM_001009931.6	c.1280G>A, p.Gly427Asp	Heterozygous	3	missense_variant	Benign
HRNR	rs7545406	NM_001009931.7	c.819C>A, p.His273Gln	Heterozygous	3	missense_variant	Benign
SPRR1B	rs3795382	NM_003125.3	c.32C>T, p.Thr11Ile	Homozygous	2	missense_variant	Benign

There were missense and synonymous variants.

Discussion

AD is the most common chronic skin disease in the world population, occurring in 20% of children and 5% of adults, and the early onset of the disease is related to genetic factors.[15] Cork MJ. et al, found that internal or hereditary factors cause the underdevelopment of skin barrier function in children. Thus, the disease tends to start at a young age.[16] Pathogenic effects of some low-frequency gene polymorphisms in the population have not been fully determined. However, due to the introduction of WES analysis, a unique method of modern molecular genetics, it may be possible to identify pathology's cause in the future; it is possible to study how the characteristics of genes responsible for skin barrier function affect physiological parameters such as skin moisture, TEWL, and skin pH.

Global trends indicate that while Atopic Dermatitis (AD) predominantly occurred in developed countries lacking biodiversity at the turn of the millennium, its prevalence has increased in countries with higher biodiversity. This shift suggests that environmental factors, alongside genetic and immunological factors, play a crucial role in the manifestation of this disorder.[16] Two meta-analyses on AD genetics have been conducted in the past decade. The e first, dating back to 2010, consolidated all research related to AD conducted up to that year, reviewing 81 studies involving 46 genes associated with AD.[15] From 2016 to 2019, the second study compiled and analyzed all studies on AD and its genetic correlations, identifying 62 genes related to different stages of AD progression. Of these, the mutation of the FLG gene was found to contribute significantly to AD development in children.[19] Research conducted on Polish children revealed that AD accompanied by mutations in the FLG gene substantially increases the risk of asthma, indicating a six-fold higher predisposition. Studies on different populations have shown that various mutations in the FLG gene contribute differently to AD development. Additionally, factors beyond genetic predisposition, such as environmental factors, play a significant role in AD development.

Table 2. The gene variants in the epidermal differentiation complex identified in participant ID4

Gene	Rs number	Transcript	Variant	Allele State	Exon	Sequence Ontology	Classification
FLG	rs1243659295	NM_002016.2	c.8510C>G, p.Ala2837Gly	Heterozygous	3	missense_variant	Likely Benign
FLG	rs2338554	NM_002016.2	c.6603T>C, p.Asp2201=	Heterozygous	3	missense_variant	Likely Benign
FLG	rs66977240	NM_002016.2	c.6626A>G, p.His2209Arg	Heterozygous	3	missense_variant	Likely Benign
FLG	rs66954353	NM_002016.2	c.6695A>C, p.K2192Q	Heterozygous	3	missense_variant	Likely Benign
FLG	rs76413899	NM_002016.2	c.2509G>A, p.Gly837Ser	Heterozygous	3	missense_variant	Benign
FLG	rs117440780	NM_002016.2	c.12090G>A, p.Thr4030=	Heterozygous	3	missense_variant	Benign
FLG	rs unknown	NM_002016.2	c.1177G>T, D393Y	Heterozygous	3	missense_variant	Benign
HRNR	rs12729662	NM_001009931.3	c.8355C>T, p.Tyr2785=	Heterozygous	3	synonymous_variant	Benign
HRNR	rs41266112	NM_001009931.3	c.8343G>C, p.Gln2781His	Heterozygous	3	missense_variant	Benign
HRNR	rs41266114	NM_001009931.3	c.8337A>C, p.Ser2779=	Heterozygous	3	synonymous_variant	Benign
HRNR	rs61814932	NM_001009931.3	c.8331T>C, p.Ser2777=	Heterozygous	3	synonymous_variant	Benign
HRNR	rs41266116	NM_001009931.3	c.8325C>T, p.His2775=	Heterozygous	3	synonymous_variant	Benign
HRNR	rs76102381	NM_001009931.3	c.8315A>G, p.His2772Arg	Heterozygous	3	missense_variant	Benign
HRNR	rs74493243	NM_001009931.3	c.8313C>T, p.Ser2771=	Heterozygous	3	synonymous_variant	Benign
HRNR	rs41266118	NM_001009931.3	c.8309T>C, p.Leu2770Pro	Heterozygous	3	missense_variant	Benign
HRNR	rs61814940	NM_001009931.3	c.5996A>T, p.Gln1999Leu	Heterozygous	3	missense_variant	VUS/Weak Pathogenic
HRNR	rs61814941	NM_001009931.3	c.5642C>T, p.Ser1881Phe	Heterozygous	3	missense_variant	VUS/Weak Pathogenic
HRNR	rs76046733	NM_001009931.3	c.3270C>T, p.Gly1090=	Heterozygous	3	synonymous_variant	Benign
HRNR	rs77376932	NM_001009931.3	c.3160G>A, p.Glu1054Lys	Heterozygous	3	missense_variant	Benign
HRNR	rs7520249	NM_001009931.3	c.1991G>A, p.Arg664Gln	Heterozygous	3	missense_variant	Benign
HRNR	rs61814946	NM_001009931.3	c.1710C>T, p.Ser570=	Heterozygous	3	synonymous_variant	Benign
HRNR	rs41266134	NM_001009931.3	c.1550A>G, p.Tyr517Cys	Heterozygous	3	missense_variant	Benign
HRNR	rs6587647	NM_001009931.3	c.1474G>A, p.Gly492Arg	Heterozygous	3	missense_variant	Benign
HRNR	rs6587648	NM_001009931.3	c.1418A>G, p.Glu473Gly	Homozygous	3	missense_variant	Benign
HRNR	rs6666097	NM_001009931.3	c.1280G>A, p.Gly427Asp	Heterozygous	3	missense_variant	Benign
HRNR	rs6587649	NM_001009931.3	c.1127A>G, p.Gln376Arg	Homozygous	3	missense_variant	Benign
HRNR	rs6587650	NM_001009931.3	c.1053T>C, p.His351=	Homozygous	3	synonymous_variant	Benign
HRNR	rs6587651	NM_001009931.3	c.984C>A, p.Gly328=	Heterozygous	3	synonymous_variant	Benign
HRNR	rs7545406	NM_001009931.3	c.819C>A, p.His273Gln	Heterozygous	3	missense_variant	Benign
HRNR	-	NM_001009931.3	c.814C>A, p.Arg272=	Heterozygous	3	synonymous_variant	Benign
HRNR	rs7535536	NM_001009931.3	c.561T>C, p.His187=	Heterozygous	3	synonymous_variant	Benign
HRNR	rs6587653	NM_001009931.3	c.558C>T, p.Ser186=	Homozygous	3	synonymous_variant	Benign
HRNR	rs11204937	NM_001009931.3	c.254G>A, p.Arg85His	Heterozygous	3	missense_variant	Benign
SPRR1B	rs3795381	NM_003125.3	c.27T>C, p.Pro9=	Homozygous	2	synonymous_variant	Benign
SPRR1B	rs3795382	NM_003125.3	c.32C>T, p.Thr11Ile	Homozygous	2	missense_variant	Benign
SPRR1B	rs12195	NM_003125.3	c.162C>T, p.Pro54=	Homozygous	2	synonymous_variant	Benign

There were missense and synonymous variants

Severe AD cases are more likely to have gene alterations, particularly those affecting skin barrier function and physiological parameters. Alterations in genes within the EDC (Epidermal Differentiation Complex) may contribute to these changes. In our study, two children, ID4 and ID102, were selected as the most suitable cases for further analysis based on the questionnaire, physical examination, laboratory evaluations, and instrumental analysis from the total of 103 children included in the study. These two participants underwent WES to investigate potential genetic contributors to their disease. Several FLG gene mutations and variants in the HRNR and SPRR1B genes were identified. These findings differ from prior studies investigating genetic associations with AD, as variants in the HRNR and SPRR1B genes have yet to be commonly reported. Although both children exhibited mutations only in these three genes, with changes observed across them out of the more than 60 genes that comprise the epidermal differentiation complex (EDC), this suggests a potentially novel genetic association with disease development, highlighting the significance of these specific genes in the pathogenesis of the condition.

The EDC is a group of genes located on a specific region of chromosome 1 (1q21), which plays a crucial role in forming and maintaining the skin barrier. The proteins encoded by the EDC genes, filaggrin, cornering, and small Proline-Rich Protein 1B, contribute to the structural integrity of the skin by helping form the cornified envelope, which is a protective barrier that prevents water loss and defends against environmental pathogens. In the study by Julie Henry. et al, cornering in purified cornified envelopes was confirmed using immunoelectron microscopy and Western blot analysis following V8-protease digestion. Hornerin is a known component of the cornified cell envelopes in the human epidermis, and its decreased expression in atopic dermatitis (AD) may play a role in the impaired skin barrier function commonly seen in the disease. [20]

In our study, participant ID4 has five variants, and participant ID102 has twenty-six variants in the HRNR gene. This significant number of variants may underscore the importance of this gene in the disease's pathogenesis. SPRR1B (Small Proline-Rich Protein 1B) is a gene that codes for a protein involved in skin barrier formation. Although diseases such as cervical intraepithelial neoplasia, benign neoplasm of the anus, and lung adenocarcinoma have been linked to SPRR1B,[21]

there is limited evidence directly connecting it to atopic dermatitis (AD). Interestingly, our study identified the same missense gene variants in HRNR in two severe AD cases (benign: rs41266134, rs6666097, rs7545406, and rs77376932; VUS/Weak Pathogenic- rs61814940) and SPRR1B (benign: rs3795382). However, HRNR and SPRR1B are part of pathways related to keratinization, which is crucial in maintaining skin barrier integrity and immune responses. Though direct associations have not been widely reported, their role in these processes may still contribute to AD pathogenesis.

WES analysis revealed multiple gene variants responsible for epidermal differentiation, but FLG gene variants were found to be all heterozygous. In the predictive scoring program, these changes are judged to be less likely to cause pathology or less likely to cause significant phenotypic manifestations. Both participants, ID4 and ID102, had severe AD, and heterozygous variants of the FLG gene were detected in both. Although heterozygous variants of the FLG gene are not disease-causing, they might cause or contribute to disease severity in combination with multiple gene variants. R501X and 2282del4 mutations of the FLG gene were the most frequent in patients with ichthyosis and AD in Europeans. Still, other mutations were identified in studies conducted by Rajeshwari, et al. and Mongolia.[22-25] In 2023, Rajeshwari, et al. conducted the first study to determine the complete sequence of the FLG gene in Asia.

In this study, the complete sequence of the FLG gene was determined in 22 specimens, and the previously recorded H2507Q, L2481S, K2444E, E2398Q, and S2366T5 polymorphisms and 17 unrecorded polymorphisms were detected. The study reported that P2238N, R2239W, and V2243L mutations located on repeats 6 and 7 of the FLG gene were the most frequent (70%) in children. Td the types of mutations differed among different ethnic groups according to the research by Zolmunkh, et al. (2016) conducted in Mongolia; the sequence of the FLG gene was determined using Sanger sequencing in 46 participants with atopic dermatitis (AD) and 12 individuals with ichthyosis. The study identified the following polymorphisms (SNPs): 1150C>T, 1741A>T, 1791C>T, 2181C>G, 2191A>G, and 2263G>A.[27] We performed WES analysis in two children with the most severe clinical signs of atopic dermatitis (AD), and this study is the first in Mongolia to not only examine the entire nucleotide sequence of the FLG gene exons but also to identify and compare the nucleotide sequences of the protein-coding exons of other related

genes. The FLG gene variants detected in our study are not currently registered in the allele frequency or 1000 Genomes Project databases and are considered rare alleles. Differences in the nucleotide sequence of genes found in geographically and physiologically diverse populations are essential for disease susceptibility, drug action, and side effects. It has also been found that the genetic causes of most disorders are complete.[25] Therefore, we need to research genes referring to AD and expand the information about the genealogy of Mongolian people. Our study found alterations in multiple genes, corroborating the findings of the studies above. Further research is necessary to accumulate new information about how these can combine with other gene variants to cause disease. Variants in the FLG, HRNR, and SPRR1B genes are rare alleles that may influence skin barrier function and disease progression. The presence of heterozygous alleles suggests multiple genetic changes might contribute to disease development. These findings underscore the need for further research into the genetic and functional factors underlying AD.

This study was conducted with a case series study design, which limits the ability to infer causality. Although the analysis was performed on only two children, the significance of the study results was enhanced by the fact that the probability of disease causation was calculated using a bioinformatics program that predicts the impact of each variant differing from the nucleotide sequence of the reference gene. The strengths of our research include its foundation at the National Center of Dermatology and the selection of Mongolian children as representative participants for studying atopic dermatitis (AD).

Conclusion

Our study found alterations in multiple genes, including FLG, HRNR, and SPRR1B. Further research is necessary to accumulate new information about how these can combine with other gene variants to cause and/or contribute to AD severity. The presence of heterozygous alleles suggests multiple genetic changes might contribute to disease development. These findings underscore the need for further research into the genetic and functional factors underlying AD.

Conflict of Interest

We declare no conflict of interest.

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References

1. Williams H, Stewart A, von Mutius E, et al. Is eczema really on the increase worldwide? *J Allergy Clin Immunol.* 2008;121(4):947-954.e915. https://doi.org/10.1016/j.jaci.2007.11.004
2. Ständer S. Atopic Dermatitis. *N Engl J Med*. 2021;384(12):1136-1143. https://doi.org/10.1056/NEJMra2023911
3. Liang Y, Chang C, Lu Q. The Genetics and Epigenetics of Atopic Dermatitis-Filaggrin and Other Polymorphisms. *Clin Rev Allergy Immunol*. 2016;51(3):315-328. https://doi.org/10.1007/s12016-015-8508-5
4. Brettmann EA, de Guzman Strong C. Recent evolution of the human skin barrier. *Exp Dermatol*. 2018;27(8):859-866. https://doi.org/10.1111/exd.13689
5. Barker JN, Palmer CN, Zhao Y, et al. Null mutations in the filaggrin gene (FLG) determine major susceptibility to early-onset atopic dermatitis that persists into adulthood. *J Invest Dermatol*. 2007;127(3):564-567. https://doi.org/10.1038/sj.jid.5700587
6. Karki R, Pandya D, Elston RC, et al. Defining "mutation" and "polymorphism" in the era of personal genomics. *BMC Med Genomics.* 2015;8:37. https://doi.org/10.1186/s12920-015-0115-z
7. Dębińska A. New Treatments for Atopic Dermatitis Targeting Skin Barrier Repair via the Regulation of FLG Expression. *J Clin Med*. 2021;10(11). https://doi.org/10.3390/jcm10112506
8. Stout TE, McFarland T, Mitchell JC, et al. Recombinant filaggrin is internalized and processed to correct filaggrin deficiency. *J Invest Dermatol*. 2014;134(2):423-429. https://doi.org/10.1038/jid.2013.284

9. Mallol J, Crane J, von Mutius E, et al. The International Study of Asthma and Allergies in Childhood (ISAAC) Phase Three: A Global Synthesis. *Allergol Immunopathol*. 2013;41(2):73-85. https://doi.org/10.1016/j.aller.2012.03.001
10. Hanifin JM, Rajka G. Diagnostic Features of Atopic Dermatitis. *Acta Derm Venereol*. 1980;60(92):44-47. https://doi.org/10.2340/00015555924447
11. Severity scoring of atopic dermatitis: the SCORAD index. Consensus Report of the European Task Force on Atopic Dermatitis. *Dermatology.* 1993;186(1):23-31. https://doi.org/10.1159/000247298
12. Parra JL, Paye M. EEMCO guidance for the in vivo assessment of skin surface pH. *Skin Pharmacol Appl Skin Physiol.* 2003;16(3):188-202. https://doi.org/10.1159/000069756
13. Stefaniak AB, Plessis J, John SM, et al. International guidelines for the in vivo assessment of skin properties in non-clinical settings: part 1. pH. *Skin Res Technol*. 2013;19(2):59-68. https://doi.org/10.1111/srt.12016
14. du Plessis J, Stefaniak A, Eloff F, et al. International guidelines for the in vivo assessment of skin properties in non-clinical settings: Part 2. transepidermal water loss and skin hydration. *Skin Res Technol*. 2013;19(3):265-278. https://doi.org/10.1111/srt.12037
15. Brunner PM, Guttman-Yassky E, Leung DY. The immunology of atopic dermatitis and its reversibility with broad-spectrum and targeted therapies. *J Allergy Clin Immunol.* 2017;139(4S):S65-S76. https://doi.org/10.1016/j.jaci.2017.01.011
16. Cork MJ, Danby SG, Vasilopoulos Y, et al. Epidermal barrier dysfunction in atopic dermatitis. *J Invest Dermatol.* 2009;129(8):1892-1908. https://doi.org/10.1038/jid.2009.133
17. Auer PL, Johnsen JM, Johnson AD, et al. Imputation of exome sequence variants into population-based samples and blood-cell-trait-associated loci in African Americans: NHLBI GO Exome Sequencing Project. *Am J Hum Genet.* 2012;91(5):794-808. https://doi.org/10.1016/j.ajhg.2012.08.031
18. Myles S, Davison D, Barrett J, et al. Worldwide population differentiation at disease-associated SNPs. *BMC Med Genomics.* 2008;1:22. https://doi.org/10.1186/1755-8794-1-22
19. Martin MJ, Estravís M, García-Sánchez A, et al. Genetics and Epigenetics of Atopic Dermatitis: An Updated Systematic Review. *Genes (Basel)*. 2020;11(4):442. https://doi.org/10.3390/genes11040442
20. Henry J, Hsu CY, Haftek M, et al. Hornerin is a component of the epidermal cornified cell envelopes. *FASEB J*. 2011;25(5):1567-1576. https://doi.org/10.1096/fj.10-168658
21. Zhang Z, Shi R, Xu S, et al. Identification of small proline-rich protein 1B (SPRR1B) as a prognostically predictive biomarker for lung adenocarcinoma by integrative bioinformatic analysis. *Thorac Cancer.* 2021;12(6):796-806. https://doi.org/10.1111/1759-7714.13836
22. Morar N, Cookson WO, Harper JI, et al. Filaggrin mutations in children with severe atopic dermatitis. *J Invest Dermatol.* 2007;127(7):1667-1672. https://doi.org/10.1038/sj.jid.5700739
23. Ekelund E, Liedén A, Link J, et al. Loss-of-function variants of the filaggrin gene are associated with atopic eczema and associated phenotypes in Swedish families. *Acta Derm Venereol.* 2008;88(1):15-19. https://doi.org/10.2340/00015555-0383
24. Gupta J, Margolis DJ. Filaggrin gene mutations with special reference to atopic dermatitis. *Curr Treat Options Allergy.* 2020;7(3):403-413. https://doi.org/10.1007/s40521-020-00271-x
25. Müller S, Marenholz I, Lee YA, et al. Association of Filaggrin loss-of-function-mutations with atopic dermatitis and asthma in the Early Treatment of the Atopic Child (ETAC) population. *Pediatr Allergy Immunol.* 2009;20(4):358-361. https://doi.org/10.1111/j.1399-3038.2008.00808.x
26. Rajeshwari KA, Thomas MM, Nagaraj G. Filaggrin Gene Mutation in Pediatric Patients with Atopic Dermatitis: A Look into Indian Gene Pool, a Pilot Study. *Indian J Dermatol.* 2023;68(2):135-140. https://doi.org/10.4103/ijd.ijd_403_22
27. Narmandakh Z, Tulgaa Kh, Munkhbayar A, et al. The Determination of Filaggrin Gene Single Nucleotides Polymorphisms in Patients with Atopic Dermatitis. Cent. Asian J. Med. *Sci*. 2017;3(1):31-40. https://doi.org/10.24079/cajms.2017.01.005