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Original Article

Genetic Etiology of Ichthyosis in Turkish Patients: Next-generation Sequencing Identified Seven Novel Mutations

Türk Hastalarda İktiyozisin Genetik Nedenleri: Yeni Nesil Dizileme Yedi Yeni Mutasyon Tanımladı

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ABSTRACT

Objective: Ichthyosis is a clinically heterogeneous group of genodermatoses characterized by widespread drying and scaling of the skin. It is also a genetically heterogeneous disorder, and 67 genes associated with the disease have been identified to date. However, there are still undiscovered genes causing the disease.

Methods: We investigated 19 Turkish patients from 17 unrelated families using clinical exome sequencing or multigene panel screening.

Results: Sixteen likely pathogenic or pathogenic variants were detected in 13 unrelated patients. We identified "variant of unknown significance" alteration in only one patient. Seven novel variants were identified in *ABCA12, ALOX12B,* and *ALOXE3.* The most commonly mutated gene was *TGM1,* followed by *ABCA12* and *ALOX12B.*

Conclusions: Because of the wide genetic variability of ichthyosis, it is difficult to diagnose the disease quickly and definitively. The clinical use of next-generation sequencing (NGS) methodologies is beneficial in the diagnostic approach to ichthyosis and genetic counseling. This study highlights the underlying molecular cause of ichthyosis by determining the mutational spectrum in a cohort of 19 patients. This study is the first and largest research from Turkey using NGS that highlights all ichthyosis subtypes.

Keywords: Congenital ichthyosis, ARCI, molecular diagnosis, NGS

ÖZ

Amaç: İktiyozis, deride pullanma ve yaygın kuruma ile karakterize klinik olarak heterojen bir genodermatoz grubudur. Aynı zamanda genetik olarak heterojen bir hastalıktır ve bugüne kadar hastalıkla ilişkili 67 farklı gen tanımlanmıştır. Bununla birlikte, hastalığa neden olan hala keşfedilmemiş genler de bulunmaktadır.

Yöntemler: Klinik ekzom dizileme veya çoklu gen paneli kullanarak, akraba olmayan 17 aileden 19 Türk hastayı araştırdık.

Bulgular: Aralarında akrabalık bulunmayan 13 hastada 16 olası patojenik veya patojenik değişim tespit edildi. Sadece bir hastada "klinik önemi bilinmeyen" değişim saptadık. *ABCA12, ALOX12B* ve *ALOXE3* genlerinde yedi yeni varyant tanımlandı. En yaygın mutasyona uğramış gen *TGM1* olup, bunu *ABCA12* ve *ALOX12B* genleri izlemektedir.

Sonuçlar: İktiyozisin geniş genetik değişkenlik göstermesi nedeniyle, hastalığı hızlı ve kesin olarak teşhis etmek zordur. Yeni nesil dizi analizi (NGS) metodolojilerinin klinik kullanımı, iktiyozisin genetik danışmanlığı ve tanısal yaklaşımında faydalıdır. Bu araştırma, 19 hastadan oluşan bir kohortta mutasyon spektrumunu belirleyerek iktiyozisin altta yatan moleküler nedenlerini açıklamaktadır. Bu çalışma, NGS yöntemi ile tüm iktiyozis alt tiplerini araştıran, Türkiye'deki ilk ve en büyük araştırmadır.

Anahtar kelimeler: Konjenital iktiyozis, ARCI, moleküler tanı, NGS

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INTRODUCTION

Ichthyosis is a genetically and phenotypically heterogeneous group of disorders characterized by hyperkeratosis of varying degrees, widespread scaling, and skin dryness[1]. Ichthyosis has been classified into two main categories, namely, the syndromic and non-syndromic forms. Clinical manifestations are limited to the cutaneous structures in non-syndromic forms. Autosomal dominant, autosomal recessive, or X-linked recessive inheritance patterns can be seen[2]. Thus far, as many as 67 genes have been identified and related to distinct types of ichthyosis[3].

The most common and relatively mild form is ichthyosis vulgaris[4]. Regarding incidence, it is followed by X-linked recessive ichthyosis and autosomal recessive congenital ichthyosis (ARCI), respectively. ARCI includes the heterogeneous group of Mendelian cornification disorders. ARCI results from mutations in at least 13 genes[5]. ARCI has three major subgroups: Harlequin ichthyosis (HI), lamellar ichthyosis (LI), and congenital ichthyosiform erythroderma (CIE). Pleomorphic ichthyosis can be defined as the fourth subgroup of ARCI[6]. Other forms of ichthyosis include congenital reticular ichthyosiform erythroderma, bathing suit ichthyosis, and ichthyosis with confetti.

HI is the most severe and rare form of ARCI caused by biallelic deletions or loss-of-function mutations in the *ABCA12* gene[7]. Biallelic pathogenic variants of TGM1, NIPAL4, ALOXE3, ALOX12B, PNPLA1, CYP4F22, LIPN, and CERS3 are responsible for the LI and CIE forms[8].

Next-generation sequencing provides a specific molecular diagnosis in most heritable disorders with extensive genetic heterogeneity[9]. Because of the broad clinical and genetic variabilities of ichthyosis, diagnosing the disorder quickly and definitively is challenging. Molecular approaches are beneficial in diagnosing and genetic counseling this group of diseases. In addition, studies on the predisposition of ichthyosis to skin cancer are increasing. There are studies on cancer susceptibility, especially in FLG mutations[10]. Therefore, genetic diagnosis with molecular methodologies has become more important for complete genetic counseling in patients with ichthyosis.

This study highlights the underlying molecular causes of ichthyosis in 19 Turkish patients, and 17 of them were unrelated.

MATERIALS and METHODS

Patients

This study was approved by the Institutional Ethics Review Committee of University of Health Sciences Turkey, Diskapi Yildirim Beyazit Training and Research Hospital (decision no: 121/06, date: 04.10.2021). Written informed consent for the use of any additional related information was obtained from all patients or their parents for enrollment in the study and publication of data. This study included all 19 patients with ichthyosis findings referred to us between 2017 and 2020. Family history, dysmorphic disorder examination, and pedigree data were evaluated.

The ichthyosis gene panel, including *ABCA12, TGM1, NIPAL4, ALOXE3, ALOX12B, PNPLA1, CERS3, CYP4F22, LIPN,* and *LIPH,* was studied in 14 patients, and clinical exome sequencing (CES) was applied in three patients (patients 9-11). In two patients, the mutation region detected in siblings with the same disease findings was studied by Sanger sequencing (patients 1 and 7).

DNA Sequencing and Variant Classification

Peripheral blood samples were collected in ethylenediaminetetraacetic acid tubes, and DNA isolation was performed using the Thermo Scientific DNA isolation kit according to the manufacturer's standard procedure. The DNA samples were quantified with a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific Inc., MA, USA).

Fourteen patients were analyzed with a custom-made ichthyosis gene panel comprising 10 genes using an Ion Torrent S5 platform (Thermo Fisher Scientific). CES was performed on an Illumina MiSeq platform (Illumina Inc., USA). CES data were analyzed on the Sophia DDM software (SOPHiA Genetics, Saint-Sulp, Switzerland). All genomic variants identified were evaluated using Ensembl Genome Bowser. Consistent with the American College of Medical Genetics and Genomics (ACMG) and the Society for Molecular Pathology, variants were classified as pathogenic, possibly pathogenic, variant of unknown significance (VUS), possibly benign, and benign[11]. Pathogenic, likely pathogenic, and VUS variants were reported in the study. No statistical analysis was performed in our study.

RESULTS

The mean age was 22 (range, 2-54) years. There were more female patients (n=11, 57%) than male patients (n=8, 43%). The majority of the observed genomic variants were

missense (87%), and frameshift and splice site alterations or variants were seen in only two patients.

In this study, 17 unrelated patients underwent gene panel or CES between January 2017 and 2021. Sixteen likely pathogenic or pathogenic variants were detected in 13 patients, of which seven novel variants were identified in four patients. The novel variants were observed in ABCA12, ALOX12B, and ALOXE3. VUS alteration was detected in only one patient. All genomic variants identified are presented in Table 1. No variants were identified in four patients related to their phenotype. We obtained a high diagnosis rate (70%) compared with previous studies. The twin brother of patient 1 and the sister of patient 7 were evaluated by Sanger sequencing, and they were found to have the same mutation. The parents of patient 3 were also assessed to detect carriers. The consanguinity rate was 52% (9/17).

All variants were detected in eight known ichthyosis genes, including *ABCA12, ALOX12B, ALOXE3, FLG, KRT10, NIPAL4, PNPLA1,* and *TGM1.* The most commonly mutated gene was *TGM1,* followed by *ABCA12* and *ALOX12B.* Eight patients had mutations in these three genes, accounting for 40% of all patients.

DISCUSSION

Ichthyosis is related to mutations in over 60 genes that encode essential proteins for normal physiological skin barrier function[12]. Thus far, studies related to ichthyosis have been published in different countries, but very few studies focused on the mutation spectrum of ichthyosis

Table 1. Mutations identified through next-generation sequencing.

Patient ID	Gender	Age	Consanguinity	Gene	Mutation	Zygosity	Consequence on protein	ACMG	Novelty
1	M	15	+	*ALOX12B*	c.811G>A p.(Gly271Ser)	Homozygous	Missense	Likely pathogenic	Novel
2	F	6	+	*TGM1*	c.1303T>C p.(Phe435Leu)	Homozygous	Missense	Pathogenic	–
3	M	35	–	*ABCA12*	c.4414C>T p.(Arg1472Cys) c.4268G>A p.(Cys1423Tyr)	Compound heterozygous	Missense Missense	Likely pathogenic Likely pathogenic	Novel Novel
4	F	12	+	*TGM1*	c.1166G>A p.(Arg389His)	Homozygous	Missense	Pathogenic	–
5	F	25	+	*PNPLA1*	c.301A>G p.(Arg101Gly)	Homozygous	Missense	Likely pathogenic	–
6	F	30	+	*ALOX12B*	c.1463G>A p.(Arg488His)	Homozygous	Missense	Pathogenic	–
7	F	54	+	*NIPAL4*	c.527C>A p.(Ala176Asp)	Homozygous	Missense	Pathogenic	–
8	M	2	+	*TGM1*	c.1303T>C p(Phe435Leu)	Homozygous	Missense	Pathogenic	–
9	M	34	+	*KRT10*	c.1447T>G p.(Ser483Ala)	Heterozygous	Missense	VUS	–
10	F	11	–	*FLG*	c.4271_4272delAA p.(Lys1424Argfs*25)	Heterozygous	Frameshift	Pathogenic	–
11	F	44	–	*ALOXE3*	c.749-1G>A c.1377C>A (p.Asp459Glu)	Compound heterozygous	Splicing Missense	Pathogenic Likely pathogenic	Novel Novel
12	M	12	–	*ABCA12*	c.1A>G (p.Met1?) c.6898T>C p.(Phe2300Leu)	Compound heterozygous	Missense Missense	Pathogenic Likely pathogenic	Novel Novel
13	F	8	+	*TGM1*	c.1166G>A p.(Arg389His)	Homozygous	Missense	Pathogenic	–

ACMG: American College of Medical Genetics and Genomics, Consanguinity +: Father and mother are consanguineous, Consanguinity –: Father and mother are nonconsanguineous, M: Male, F: Female

in Turkey. This study focused on the molecular cause of ichthyosis by determining the mutational spectrum in a cohort of 19 patients. To our knowledge, this is the first and largest study from Turkey using NGS that highlights all ichthyosis subtypes.

In patients with ARCI, the most commonly mutated genes are *TGM1* and *NIPAL4*, which are responsible for approximately 50% of all mutations[13]. In this study, *TGM1* is the most frequently mutated gene in correlation with previous studies and literature (4/17, 23%). However, the most common mutation after *TGM1* was found in *ABCA12* and *ALOX12B*. These three genes were responsible for 40% of patients with ichthyosis. Therefore, *TGM1*, *ABCA12*, and *ALOX12B* mutations should not be ignored in patients from Turkish population diagnosed with ichthyosis, especially ARCI.

ALOX12B and ALOXE3 encode the epidermal lipoxygenases 12R-lipoxygenase and lipoxygenase 3, respectively[14]. Approximately 88 pathogenic variants have been identified in ALOX12B, of which 64% are missense variants. Mutations within the gene are unevenly distributed, and according to Hotz et al.[15], mutations were most frequently observed in exon 9. The least mutation frequency was observed between exons 3 and 6. However, functional studies on this subject are insufficient; thus, future studies may provide information about hotspot mutation regions. Most cases have mild erythrodermic ichthyosis, and only a few patients present with severe erythroderma. Although a clear genotype-phenotype correlation has not been determined in ALOX12B, there are opinions that mutations in evolutionarily conserved regions cause more severe disease[16].

Patient 1 presented with erythroderma, palmoplantar keratoderma, and dry skin. A novel homozygous missense mutation (c.811G>A) in exon 7 of ALOX12B was identified. We detected the same variant in his twin brother by Sanger sequencing (Figure 1). According to the ACMG guidelines, this variant was likely pathogenic with increased segregation data. In patient 6, we also detected a mutation in ALOX12B, who had similar findings to patient 1, and both have relatively mild disease. In patient 6, NGS revealed homozygous c.1463G>A mutation in exon 11 of the gene, as previously reported. The pathogenic variants detected in both patients were not found in the hotspot region. New studies are needed to make a precise genotype-phenotype correlation.

Most of the truncation or deletion mutations in the conserved region of ABCA12 lead to a severe form of ARCI (HI). Missense mutations are primarily responsible

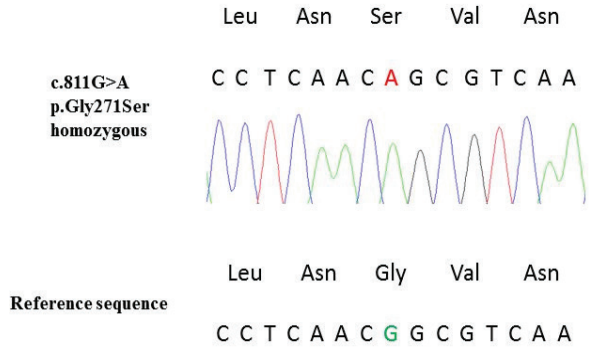

Figure 1. Results of ALOX12B Sanger sequencing in the twin brother of patient 1. A novel homozygous missense mutation was identified.

for LI and CIE subtypes[17]. In patient 3, a novel compound heterozygous mutation was detected in the *ABCA12* gene. By NGS, a heterozygous c.4268G>A (p.Cys1423Tyr) variant in exon 29 and a heterozygous c.4414C>T (p.Arg1472Cys) variant in exon 30 were found. The segregation of these variants in a patient's family was confirmed by Sanger sequencing. The family segregation study showed that the c.4268G>A variant was inherited from the mother, and the c.4414C>T variant was inherited from the father. According to the ACMG guidelines, these variants were classified as likely pathogenic. Consistent with previous studies, our patients with missense variants have no severe clinical conditions.

FLG mutations cause ichthyosis vulgaris, the most common type of ichthyosis[4]. Besides, loss-of-function mutations in FLG are crucial genetic risk factors for atopic dermatitis and have been associated with more severe and early-onset disease[18]. CES analysis revealed a heterozygous frameshift pathogenic mutation in FLG of patient 10, who was followed up with immunoglobulin E-associated atopic dermatitis. This case shows that FLG mutations should not be overlooked, especially in patients with severe atopic dermatitis.

Although studies on the relationship between ichthyosis and skin cancer and their molecular mechanisms have increased in recent years, it will continue to be defined in the future[19]. As a result of loss-off function mutations in FLG, FLG deficiency in the epidermis leads to decreased protection against UV exposure and an increased risk of DNA damage and neoplasia. Specifically, an increased risk of basal cell carcinoma and squamous cell carcinoma was reported[20].

Therefore, these patients should be offered regular examinations for skin cancer. Discoveries in ichthyosis genetics will provide a better understanding of skin cancer predisposition.

CONCLUSIONS

NGS methodologies are very useful in the diagnostic approach to ichthyosis. In addition, the detection of heterozygous carriers, especially in consanguineous families, allows preimplantation genetic diagnosis. Our study unravels the molecular etiology of Turkish patients with ichthyosis and contributes to broadening the mutation spectrum in ABCA12, ALOX12B, ALOXE3, FLG, KRT10 NIPAL4, PNPLA1, and TGM1.

Ethics

Ethics Committee Approval: This study was approved by the Institutional Ethics Review Committee of University of Health Sciences Turkey, Diskapi Yildirim Beyazit Training and Research Hospital (decision no: 121/06, date: 04.10.2021).

Informed Consent: Written informed consent for the use of any additional related information was obtained from all patients or their parents for enrollment in the study and publication of data.

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Author Contributions

Surgical and Medical Practices: H.S., I.S., M.G., T.B., Concept: H.S., Design: H.S., Data Collection and/or Processing: H.S., I.S., N.D., M.G., T.B., Analysis and/or Interpretation: H.S., I.S., N.D., T.B., Literature Search: H.S., Writing: H.S.

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