Identification of a homozygous mutation in the ABHD5 gene in two non-consanguineous Iranian-Azari Turkish families with Chanarin-Dorfman syndrome

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Short Report

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Abstract

Background: Chanarin-Dorfman Syndrome (CDS) is a rare autosomal recessive type of Non-bullous Congenital Ichthyosiform Erythroderma (NCIE) caused by mutations in the *ABHD5*.

Methods: Whole-exome sequencing (WES) was performed on a 4-year-old boy born to a non-consanguineous marriage. This boy had developed skin dryness and scaling. Two other individuals from a related family with non-consanguineous marriage also shared the same clinical manifestations. DNAs were extracted from the blood samples of all the three affected individuals and subjected to PCR-Sanger sequencing to confirm the WES results.

Results: WES data analysis identified a single nucleotide (C) insertion at the position of 594 (c.594dup) at exon 4 of the *ABHD5* gene in homozygous state. This mutation, disturbing the vital domain (α/β hydrolase fold domain) of the encoded protein and also not being observed in our control population nor being reported in any other population database, is classified as a pathogenic mutation. PCR-Sanger sequencing data analysis confirmed the WES result and also showed that the two other affected individuals carried the same mutation in homozygous state. Parents were carriers for the mutation.

Conclusions: An insertion (c.594dup) mutation resulting in a frameshift and creating an early stop codon at the *ABHD5* gene and ultimately removing an essential domain of the encoded protein, is associated with dry skin, hepatomegaly, subsequent elevations in liver enzymes, and the buildup of lipid vacuoles in white blood cells. Observed different clinical manifestations in two studied-family members could be due to modifier genes and/or the gender impact.

Background

The epidermis is in constant touch with the external environment, posing a danger of infection to organisms. However, it has a strong permeability barrier, which significantly inhibits the entry of external pathogens, allergies, and other hazardous substances and inhibits internal water transpiration. Since these physiological functions are crucial, alterations in this permeability barrier can result in various skin diseases, including ichthyosis and atopic dermatitis [1, 2].

The strong hydrophobicity of lipids makes them ideal for use in permeable barriers. Indeed, the multilayered structure of lipids (lipid lamellae) seen in the epidermis's outermost cell layer, the stratum corneum, is critical for creating the skin's permeability barrier. Ceramides, cholesterol, and free fatty acids are the primary components of lipid lamellae. Ceramides are abundant in the epidermis; among them, omoega-O-acylceramide (acylceramide), an epidermis-specific ceramide species, is required to establish a skin permeability barrier [1–3].

The production of acylceramide requires several steps, including fatty acid elongation, hydroxylation, and formation of an amide bond between long-chain base and esterification, which are controlled by serval

genes like *CYP4F22*, *CERS3*, and *PNPLA1* genes. Mutations in these genes cause autosomal recessive congenital ichthyoses [4–6].

Chanarin-Dorfman Syndrome (CDS)(OMIM 275630), an extremely rare autosomal recessive variant disease, is caused by mutations in the Alpha-Beta-Hydrolase Domain-Containing-5 (*ABHD5*) gene, also known as Comparative Gene Identification-58 (CGI-58) [7]. This gene encodes a protein that functions as an acyltransferase in the production of phosphatidic acid and as a coactivator for adipocyte triglyceride lipase (ATGL or PNPLA2). Deficiencies in the function of this protein results in ichthyosis and lipid storage disorder characterized by the accumulation of lipid vacuoles in neutrophils which was described initially by Dorfman in 1974 [8, 9].

A recent study demonstrated that the mutations in *ABHD5* gene identified in patients with Chanarin–Dorfman syndrome impaired *ABHD5*'s ability to enhance PNPLA1-dependent acylceramide synthesis [10].

Here we report a homozygous variant in the gene *ABHD5* in two related families with non-consanguineous marriage, with the accumulation of lipid vacuoles in white blood cells (Jordan's Syndrome) and widespread and persistent thick and dry skin.

Methods

A 4-year-old patient with NBCIE disease was referred to the Medical Genetics Lab for a genetic study using whole-exome sequencing (WES). Pedigree analysis showed that there were two other members in the relatives of this family affected with the same disease. All patients were born to non-consanguineous families. Upon written consent, blood samples were taken from all affected members of the families on EDTA tubes. DNA of proband (the four-year-old Iranian-Azeri Turkish boy) was extracted, quantified, and subjected to WES analysis as described previously [11, 12]. In brief, Agilent SureSelect Human All Exon V7 Target Enrichment kit was used to perform library preparation. The enriched library was sequenced on an Illumina NovaSeq 6000 platform.

The FastQC tool was used to verify the quality of the Fastq files. Burrows-Wheeler Aligner (BWA-mem) was used to map paired-end 150-bp sequencing reads to the UCSC human reference genome (GRCh37/hg19 and GRCh38/hg38 assembly) [13]. The Picard and Trimmomatics V0.39 tools were used to eliminate duplicates and low-quality bases (Qbase 20) [14]. For sorting and indexing bam files, Samtools was utilized [15]. Parallel identification of single nucleotide variations (SNVs) and tiny insertions or deletions (Indels) was performed using the Genome Analysis Toolkit (GATK-version 40205.0) [16] and DeepVariant 1.1.0 [17]. The Wannovar and SnpEff tool were used to annotate the variants [18, 19].

A variant filtering workflow developed in-house was used to identify disease-causing variations in accordance with ACMG and Sherloc standards [20]. Variations were initially filtered according to their minor allele frequency (MAF) in several population databases (dbSNP, 1000 Genomes, ExAc database, gnomAD, and Iranome database). MAF > 0.05 was used to exclude common variations. The exon flanking

regions were retained while the deep intronic, upstream/downstream synonymous variations were eliminated. The remaining variants were then prioritized based on their functional consequences, Clinvar results, and numerous lines of bioinformatics predictions to assess the effect of the mutations.

After identifying the disease-causing mutation in the proband, Sanger sequencing was undertaken on the patient and two other affected members of the related family using specific primers designed for *ABHD5* on ABI 3500 Genetic Analyzer to validate the result (Applied Biosystems, Foster City, CA, United States).

The NCBI reference sequence for the human *ABHD5* protein (NP 001342115.1) was used to determine the effect of the identified mutation on ABHD5 function. I-Tasser, an in silico protein modeling tool (https://zhanggroup.org/), was used to construct 3D models of *ABHD5* wild-type and *ABHD5* (c.594dupC) [21]. The secondary structure and the Pfam domain of the wild-type and mutant proteins were predicted by CLC genomic workbench version 21.

Results

Case presentation

The proband was born to a non-consanguineous family (Figure 1). He was delivered at 39 weeks gestation with normal pregnancy history. At birth he was covered with a taut shiny membrane but in a few days this membrane gradually dried and peeled off and mild dryness and scaling left behind. Skin dryness and scaling were detected at four months, which gradually intensified. He was hospitalized for a high fever at six months after taking Tetanus vaccine, diphtheria, pertussis, hepatitis B, Hemophilus influenzae, and Polio vaccines. During his hospitalization, his hepatosplenomegaly was recognized. He was evaluated for storage disease such as Niemann-Pick disease, Gaucher disease, pompe disease, and rheumatologic disease. But no positive findings were found in favor of these diseases. Following a diagnosis of elevated liver enzymes and hepatosplenomegaly on ultrasound, the patient underwent liver biopsy and was diagnosed with micronodular cirrhosis with mild macrovesicular steatosis (25 %). His liver AST and ALT enzyme levels were two to three times of the normal limit. However, ALP and bilirubin levels were normal. The patient's neuromuscular development was normal, and no muscle weakness was noticed. The blood CPK and Aldolase levels were slightly elevated. At the age of two, he had EMG-NCV with no myopathic or neuropathic indications. At this age the eye and heart examinations were normal. Other blood cholesterol, Triglyceride, and renal function tests were normal. He was diagnosed with apparent ichthyosis at age four, characterized by generalized scaly skin, particularly on the palm (Figure 2).

Two other members of the related family were also diagnosed with the same disease (Figure 1, Family II). They were a 20-year old girl and her 13-year old brother, born to a family with non-consanguineous marriage. The second case (20-year-old girl) diagnosed with ichthyosis after being born with congenital widespread skin dryness. No further clinical manifestations were noted throughout childhood. Neuronal development was normal, and there was no evidence of hypotonia. She exhibits a little ectropion. This exposes and irritates the inner eyelid surface. The levels of the AST and ALT enzymes were two to three

times those found in healthy persons, although this was controlled. A slight increase in blood bilirubin was also seen. On ultrasound, the liver was normal in size but the echogenicity was relatively coarse. The patient did not undergo a liver biopsy for further investigation. Jordan's anomaly was also validated by accumulating lipid vacuoles in white blood cells (Figure 3).

The third case was the younger brother of the second case. He was 13 years old when he was diagnosed. At birth he had a slight scaling on the scalp, but it was less severe than the second case. Afterward, his skin dryness began to spread to other areas of the body (Figure 4). Additionally, he was diagnosed with ichthyosis and began using emollient creams. Similar to the first case, there was a significant rise in liver enzymes (AST and hepatomegaly and increased liver echogenicity on ultrasound). The patient underwent liver biopsy for additional study due to mild hepatomegaly. Steatosis was shown in around 70% of parenchymal cells, most of which were macrovascular. There were no signs of interface hepatitis or confluent necrosis (liver steatosis). Neither Ectropion nor entropion was seen in this case and neuromuscular examinations were normal (Table 1).

Table 1

Clinical data and molecular analysis of the patients with CDS

No.	Age	Gender	Phenotype	AST/ALT	Levels of Ch,TG,	WBC anomaly
1	4y	Male	NLSD	3 x normal	Normal	Jordan´s anomaly
2	20y	Female	NLSD	AST: 28 U/L ALT: 30 U/L	Normal	Jordan´s anomaly
3	13y	Male*	NLSD	AST: 97 U/L ALT: 124 U/L	Normal	Not tested

AST; aspartate aminotransferase, ALT; alanine aminotransferase

Genetic investigation

WES analysis revealed a homozygous C insertion in exon 4 of the *ABHD5* gene (c.594dupC), causing a frameshift and premature termination at amino acid 209 (p.Arg119Glnfs*11) (Figure 5). Primers were designed from the region of interest, and the mutation was verified using Sanger sequencing (Figure 5). The un-relative parents were heterozygous for the mutation. Sanger sequencing of two other patients (cases number 2 and 3) using the designed primers, revealed that these patients carrying homozygous mutations in *ABHD5* gene.

The *ABHD5* gene contains seven exons and is located on chromosome 3. This gene codes for a 349 amino acid protein. The molecular weight of the wild-type protein is 39.095 KD. The Pfam domain search for this protein discovered an alpha/beta hydrolase fold domain that begins at amino acid 76 and ends

at amino acid 334. This domain contains two amino acid residues involved in the interaction between ATGL and perilipin (Q130 and E206). *ABHD5* activates PNPLA1 during the epidermal barrier development, hence determining the generation of O-acylceramide from hydroxyceramide. *ABHD5* acts as an ATGL coactivator in various organs such as the liver, muscle, and immune cells, including TAG hydrolysis. The mutant protein contains 208 amino acids and has a molecular weight of 23.292 KD. This mutation alters the alpha/beta hydrolase fold domain, which is required for the enzyme to operate correctly (Figure 6).

The final models for the wild-type and mutant protein were predicted by the I-TASSER tool. Each model's confidence is quantified using a C-score generated from the importance of threading template alignments and the convergence parameters of structure assembly simulations. The C-score is typically between (-5, 2), with a higher C-score indicating a more confident model and vice versa**.** Figure 6, D illustrates the first model with the highest C-score for each WT and mutant protein. Eliminating the ligand-binding site residues (266, 300, 301, 327, 328) and alpha/beta hydrolase fold domain altered the protein's structure.

Discussion

CDS is a neutral lipid disorder that impacts the skin, eyes, central nervous system, skeletal muscle, liver, and bone marrow. While the extracutaneous manifestations vary in appearance and severity, ichthyosiform erythroderma is often present in all patients, beginning at birth [10]. Ichthyosis is characterized by widespread scaly lesions with an erythematous background in CDS. However, CDS can mimic other skin diseases with erythema and scales [1]. A case of erythrokeratodermia variabilis-like CDS with normal skin patches alternating with erythematous scaly patches has been reported.

Due to the variable clinical appearance of the skin, it took a long time to get an accurate diagnosis. Liver involvement and hyperlipemia are frequent findings in CDS, occurring in more than 80% of patients. Currently, no specific treatment exists for CDS. However, a low-fat diet supplemented with medium-chain triglycerides (MCT) has been shown to reduce hepatomegaly and normalize liver enzymes, especially when initiated early and combined with vitamin E and ursodeoxycholic acid [22].

Ohno et al. explored the molecular mechanism underlying the Chanarin-Dorfman syndrome's ichthyosis pathology. The *ABHD5* promotes acylceramide synthesis mediated by PNPLA1 [11]. It has been recently shown that mono-allelic mutations in the *ABHD5* gene causes non-alcoholic fatty liver disease (NAFLD) and emphasize the critical need for additional research on the involvement of LD problems in liver pathology [23].

In sum, an insertion mutation was found in the gene in two related and non-consanguineous families. The proband was diagnosed with CDS, and genetic testing was recommended to the second family due to the clinical similarities. Two members of the second family were also diagnosed with CDS due to the same mutation. This mutation (c.594dupC) in the ABHD5 gene results in a frameshift and a premature stop codon (p.Arg119Glnfs*11). Based on ACMG/AMP variant classification, this variant is absent from controls (PM2). A reputable source reports the variant as pathogenic (PP5) [7]. The ABHD5 protein has two main domains: hydrophobic motif and α/β hydrolase fold domain. This mutation disrupts the α/β

hydrolase fold domain, which is crucial for the proper activity of the protein. Different clinical manifestations of this mutation in afflicted family members may be due to modifier genes or gender differences.

Conclusions

Observing two families with non-consanguineous marriage harboring the same mutation, indicates the possibility of higher frequency of the mutation in the region these families reside.

Declarations

All methods were carried out in accordance with relevant guidelines and regulations or Declaration of Helsinki.

Ethics approval and consent to participate:

This project was ethically approved by University of Tabriz and written informed consent was taken from all participants.

Consent for publication:

Not applicable.

Availability of data and materials:

All data generated or analyzed during this study are included in this published article.

Competing interests:

The authors declare that they have no competing interests.

Funding:

There was no funding for the project.

Authors' contributions:

HS performed biomedical analysis and was a contributor in preparation of the manuscript. NJ analyzed WES data, designed primers and analyzed Sanger sequence. AR performed clinical examination on patients and contributed in preparation of the manuscript. MB designed the project, contributed in analyzing the WES results and was a main contributor in manuscript preparation. H.S & N.J. contributed equally to the project and data analysis.

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References

1. Akiyama M, Shimizu H. An update on molecular aspects of the non-syndromic ichthyoses. Exp Dermatol [Internet]. 2008 May 1;17(5):373–82. Available from: https://doi.org/10.1111/j.1600-0625.2007.00691.x
2. Ishikawa J, Narita H, Kondo N, Hotta M, Takagi Y, Masukawa Y, et al. Changes in the Ceramide Profile of Atopic Dermatitis Patients. J Invest Dermatol [Internet]. 2010;130(10):2511–4. Available from: https://www.sciencedirect.com/science/article/pii/S0022202X15345553
3. Kihara A. Synthesis and degradation pathways, functions, and pathology of ceramides and epidermal acylceramides. Prog Lipid Res [Internet]. 2016;63:50–69. Available from: https://www.sciencedirect.com/science/article/pii/S0163782716300078
4. Ohno Y, Kamiyama N, Nakamichi S, Kihara A. PNPLA1 is a transacylase essential for the generation of the skin barrier lipid ω-O-acylceramide. Nat Commun. 2017 Mar;8:14610.
5. Hirabayashi T, Anjo T, Kaneko A, Senoo Y, Shibata A, Takama H, et al. PNPLA1 has a crucial role in skin barrier function by directing acylceramide biosynthesis. Nat Commun [Internet]. 2017 Mar 1;8:14609. Available from: https://pubmed.ncbi.nlm.nih.gov/28248300
6. Hirabayashi T, Murakami M, Kihara A. The role of PNPLA1 in ω-O-acylceramide synthesis and skin barrier function. Biochim Biophys acta Mol cell Biol lipids. 2019 Jun;1864(6):869–79.
7. Lefèvre C, Jobard F, Caux F, Bouadjar B, Karaduman A, Heilig R, et al. Mutations in CGI-58, the gene encoding a new protein of the esterase/lipase/thioesterase subfamily, in Chanarin-Dorfman syndrome. Am J Hum Genet [Internet]. 2001/10/02. 2001 Nov;69(5):1002–12. Available from: https://pubmed.ncbi.nlm.nih.gov/11590543
8. Lord CC, Thomas G, Brown JM. Mammalian alpha beta hydrolase domain (ABHD) proteins: Lipid metabolizing enzymes at the interface of cell signaling and energy metabolism. Biochim Biophys Acta. 2013 Apr;1831(4):792–802.
9. Brown AL, Mark Brown J. Critical roles for α/β hydrolase domain 5 (ABHD5)/comparative gene identification-58 (CGI-58) at the lipid droplet interface and beyond. Biochim Biophys acta Mol cell Biol lipids. 2017 Oct;1862(10 Pt B):1233–41.
10. Ohno Y, Nara A, Nakamichi S, Kihara A. Molecular mechanism of the ichthyosis pathology of Chanarin-Dorfman syndrome: Stimulation of PNPLA1-catalyzed ω-O-acylceramide production by ABHD5. J Dermatol Sci. 2018 Dec;92(3):245–53.
11. Li H, Durbin R. Fast and accurate long-read alignment with Burrows-Wheeler transform. Bioinformatics. 2010 Mar;26(5):589–95.
12. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, et al. The Sequence Alignment/Map format and SAMtools. Bioinformatics. 2009 Aug;25(16):2078–9.

13. Li H, Durbin R. Fast and accurate short read alignment with Burrows–Wheeler transform. Bioinformatics [Internet]. 2009 Jul 15;25(14):1754–60. Available from: https://doi.org/10.1093/bioinformatics/btp324
14. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics [Internet]. 2014 Aug 1;30(15):2114–20. Available from: https://doi.org/10.1093/bioinformatics/btu170
15. Danecek P, Bonfield JK, Liddle J, Marshall J, Ohan V, Pollard MO, et al. Twelve years of SAMtools and BCFtools. Gigascience. 2021 Feb;10(2).
16. McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, et al. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res. 2010 Sep;20(9):1297–303.
17. Poplin R, Chang P-C, Alexander D, Schwartz S, Colthurst T, Ku A, et al. A universal SNP and small-indel variant caller using deep neural networks. Nat Biotechnol [Internet]. 2018;36(10):983–7. Available from: https://doi.org/10.1038/nbt.4235
18. Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. Nucleic Acids Res [Internet]. 2010 Sep 1;38(16):e164–e164. Available from: https://doi.org/10.1093/nar/gkq603
19. Cingolani P, Platts A, Wang LL, Coon M, Nguyen T, Wang L, et al. A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of Drosophila melanogaster strain w1118; iso-2; iso-3. Fly (Austin) [Internet]. 2012;6(2):80–92. Available from: https://pubmed.ncbi.nlm.nih.gov/22728672
20. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015 May;17(5):405–24.
21. Zheng W, Zhang C, Li Y, Pearce R, Bell EW, Zhang Y. Folding non-homologous proteins by coupling deep-learning contact maps with I-TASSER assembly simulations. Cell reports methods. 2021 Jul;1(3).
22. Zhang L, Wang X, Chen S, Wang S, Tu Z, Zhang G, et al. Medium-Chain Triglycerides Attenuate Liver Injury in Lipopolysaccharide-Challenged Pigs by Inhibiting Necroptotic and Inflammatory Signaling Pathways. Int J Mol Sci [Internet]. 2018 Nov 21;19(11):3697. Available from: https://pubmed.ncbi.nlm.nih.gov/30469452
23. Youssefian L, Vahidnezhad H, Saeidian AH, Pajouhanfar S, Sotoudeh S, Mansouri P, et al. Inherited non-alcoholic fatty liver disease and dyslipidemia due to monoallelic ABHD5 mutations. J Hepatol. 2019 Aug;71(2):366–70.

Figures

Figure 1

Pedigree of two linked families with Iranian Azeri-Turkish origin who have a bi-allelic *ABHD5* mutation. Note the lack of consanguineous marriages in these families. Proband was a four-year-old member of the family I. Two twenty-year-old daughter and her thirteen-year-old brother tested positive for the same mutation in the family II.

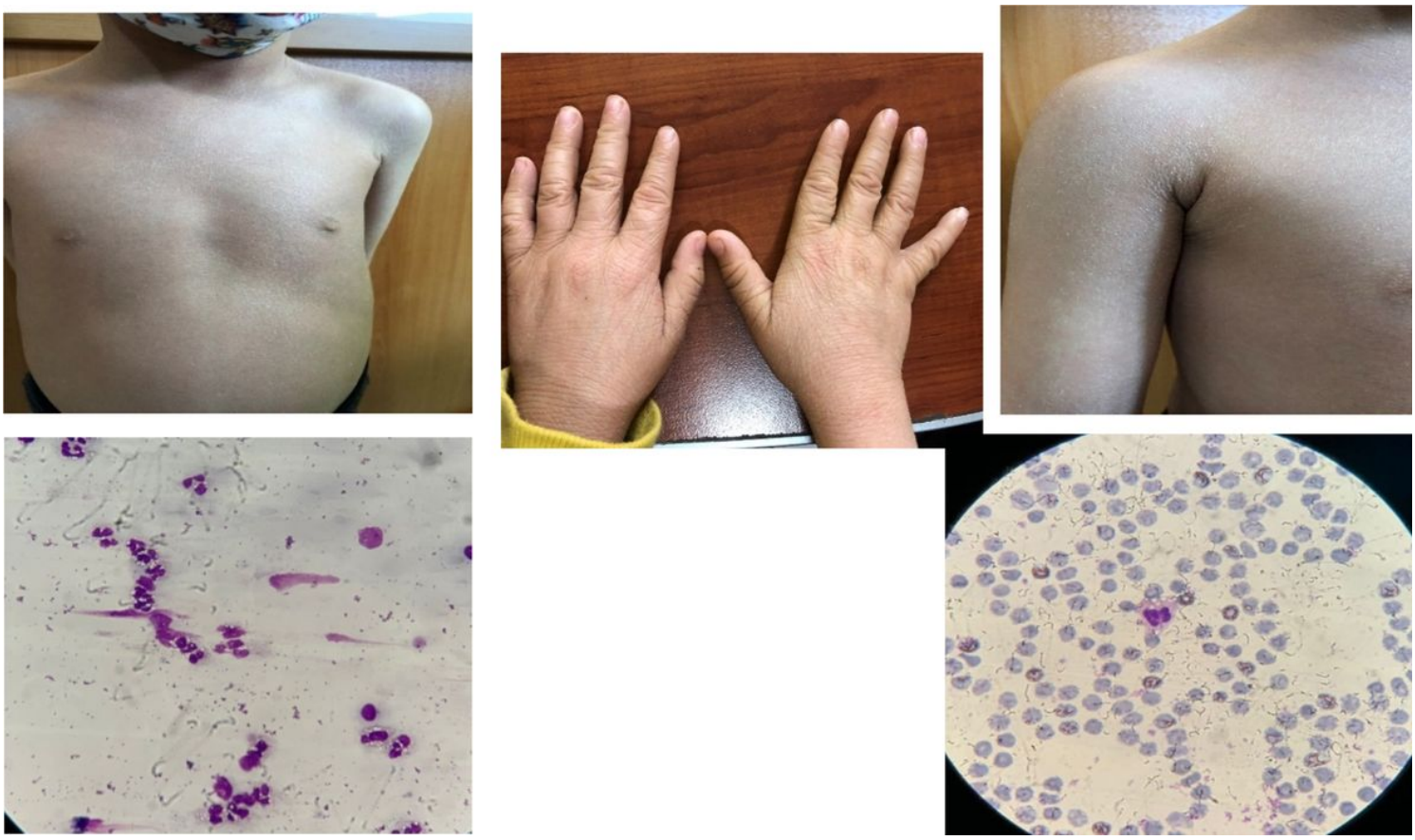

Figure 2

Clinical presentation of the first case. Intracytoplasmic vacuoles (Jordan's bodies) in neutrophil of the peripheral blood smear (May-Grunwald-Giemsa).

Figure 3

Clinical presentation of the second case. Intracytoplasmic vacuoles (Jordan's bodies) in neutrophil of the peripheral blood smear (May-Grunwald-Giemsa).

Figure 4

Clinical presentation of the third case. This patient, had a slight scaling on the scalp, but it was less severe than the second case. His skin dryness began to spread to other areas of the body

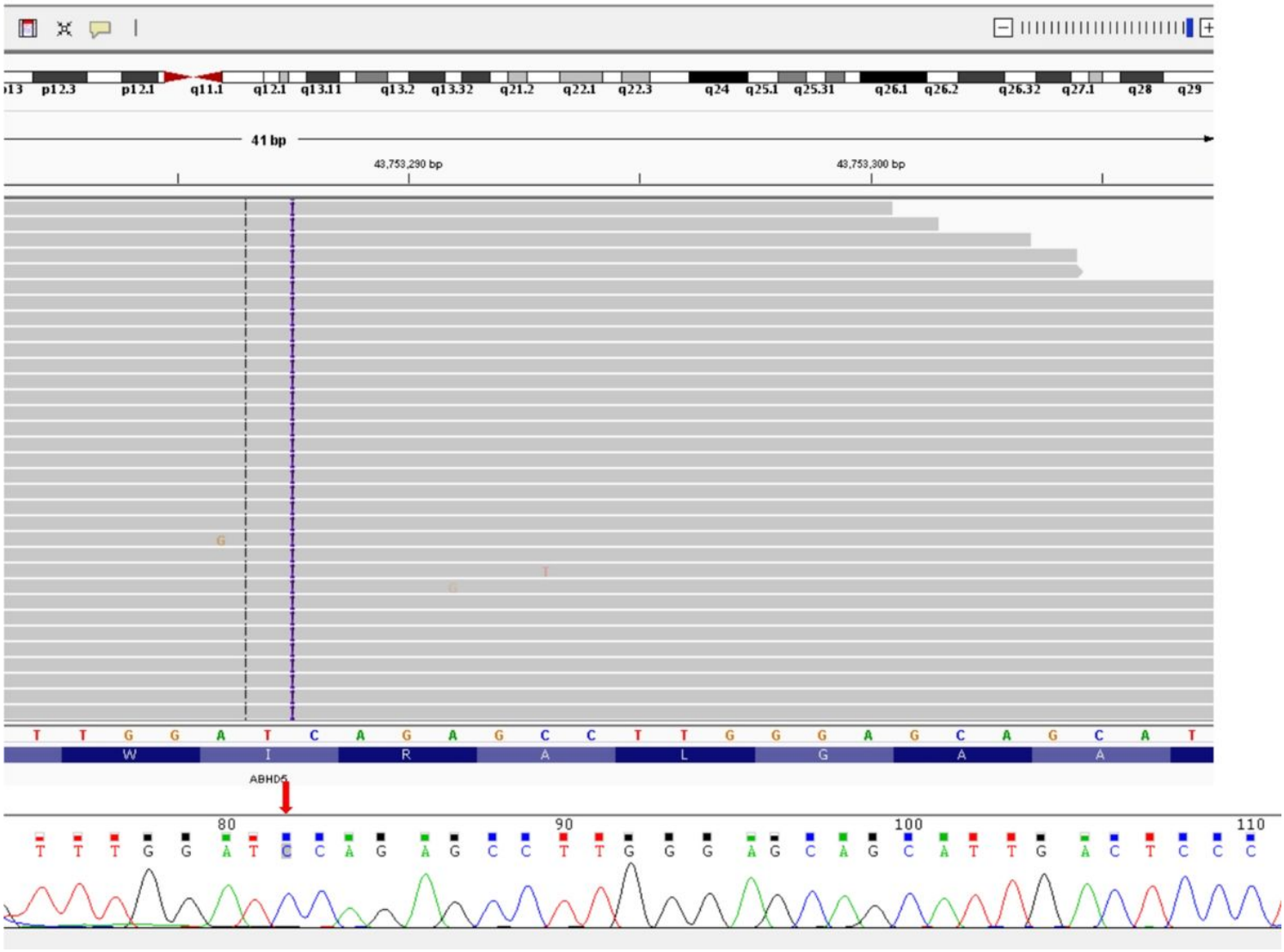

Figure 5

Exome sequencing identified a novel homozygous insertion mutation (c.594dupC) in *ABHD5* gene. The frameshift starts at codon Arg199 and the new frame ends in a STOP codon at position 11 (p.Arg199Glnfs*11) (top figure).

Sanger sequencing was utilized to confirm the result obtained from WES experiment (bottom figure).