Recurrent N209* ABHD5 mutation in two unreported families with Chanarin Dorfman Syndrome

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Abstract

ABHD5 protein is widely involved in lipid and energy homeostasis. Mutations in the ABHD5 gene are associated with the onset of Neutral Lipid Storage Disease with Ichthyosis (NLSDI), historically known as Chanarin Dorfman Syndrome (CDS). CDS is a rare autosomal recessive lipid storage disease, characterized by non-bullous congenital ichthyosiform erythroderma (NCIE), hepatomegaly and liver steatosis. Myopathy, neurosensory hearing loss, cataracts, nystagmus, strabismus, and mental impairment are considered additional findings. To date, 151 CDS patients have been reported all over the world. Here we described two additional families with patients affected by CDS from Turkey. Our patients were a 42 and 22-years old men, admitted to the Hospital for congenital ichthyosis. Hepatic steatosis and myopathy were also detected in both patients. ABHD5 molecular analysis revealed the presence of N209* mutation. Our data enlarge the cohort of CDS patients and provide a revision of muscle clinical findings for this rare inborn error of neutral lipid metabolism.

Key Words: Lipid metabolism defect; lipid droplets; Chanarin Dorfman syndrome; ABHD5 mutations; myopathy.
identified in 52% (21/40) of patients from Turkey and in 16% of all CDS patients (24/151). Here, we report the molecular and clinical characterization of two additional Turkish families with patients affected by CDS. Genetic analysis revealed the N209* mutation, confirming the diagnosis.

**Materials and Methods**

Dermatological, neurological, hepatic and muscle investigation were performed. Clinical data were collected following ethical principles reported in the Declaration of Helsinki. Peripheral blood smears were stained with May-Grünwald Giemsa to reveal Jordan’s bodies in neutrophils using a light microscopy. Genomic DNA was extracted from blood samples, and ABHD5 coding regions and their intron/exon boundaries were examined by PCR analysis. Primer sequences and amplification protocol were previously described. Finally, all PCR products were purified and sequenced (Eurofins Genomics, DNA sequencing service). Molecular analysis was carried out after obtaining the written, informed consents.

**Results and Discussion**

**Patient 1** is a 42-year-old man admitted to Adana outpatient clinic for congenital ichthyosis. Due to his complaints, he only used topical moisturizers. His parents were not consanguineous, and he had 9 siblings (Figure 1a). His mother and two siblings had congenital ichthyosis. Dermatological examination of patient revealed widespread ichthyosis containing facial region, trunk, extensor and flexural regions, and scalp. Individual scale over the trunk was white, fine, translucent and semi-adherent, while that on limbs and face was grey brown, larger in size, polygonal and adherent. There were no bullous lesions. Involvement of palms and soles, dental anomaly and nail dystrophy were absent. Intracytoplasmic vacuoles (Jordan’s anomaly) were detected in neutrophils on peripheral blood smear stained with May-Grünwald Giemsa (Figure 2a-b). Muscle strength, mental status and neurologic examination were normal, and the patient denied any hearing or visual problem. Liver transaminases, creatine kinase (CK) and serum triglyceride (TG) levels were elevated (alanine transaminase: 53 U/L [normal 5-35 U/L]; aspartate transaminase: 46 U/L [normal 5-17 U/L]; CK: 393 U/L [normal 22-200 U/L]; TG: 162 mg/dL [normal 35-130 mg/dL]). Abdominal ultrasonography was compatible with grade I hepatic steatosis. Finally, electromyographic (EMG) examination was compatible with myopathy. After 3 months of systemic acitretin treatment (25 mg/day), skin lesions of patient improved (Figure 2c-f). In agreement of what reported in other CDS patients, acitretin treatment led to a remarkable improvement in the appearance of the skin without any deleterious effect on liver function. Acitretin is a synthetic retinoid commonly used to treat severe skin problems, including ichthyosis. As a retinoid, it influences cellular division and differentiation of stratified structures of the epidermis. It exerts anti-inflammatory effect and decreases epidermal cell proliferation, inducing differentiation, and cornification.

**Patient 2** is a 22-year-old man admitted to Adana hospital because of suffering from congenital ichthyosis. His parents were relatives. He had two siblings, a sister and a brother, without clinical manifestations (Figure 1b). Dermatological examination showed features similar to patient 1: widespread ichthyosis involving facial region, trunk, extensor and flexural regions, and scalp. On the trunk region there were white, fine, translucent and semi-adherent scales, while limbs and face grey brown, large, polygonal and adherent scales (Figure 3a-e). Also in this case, there were neither bullous lesions nor involvement of palms and soles and

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**Fig 1.** Characterization of two patients with CDS. Pedigree of CDS family 1 (a) and 2 (b). Localization of mutation in the ABHD5 gene identified in patient 1 and 2 (c).
nail dystrophy. The investigation of TAG abnormal storage displayed Jordan’s bodies in granulocytes (Figure 3f-h). Evaluation of muscles highlighted normal strength, while EMG examination was consistent with myopathy. Hearing or visual, as well as mental or neurologic problems were not detected. Systemic evaluation was compatible with grade I hepatic steatosis and splenomegaly (153 mm), and laboratory tests showed an increase of liver enzymes (alanine transaminase: 82 U/L; aspartate transaminase: 59 U/L), CK (438 U/L) and serum TGs (178 mg/dL). In the examination of the oral mucosa, periodontitis was detected and panoramic radiograph revealed periodontal recession (Figure 3i-j). A low triglyceride diet supplemented with MCT oil was recommended to ameliorate hepatic and skin conditions, as previously reported in many CDS patients.\textsuperscript{17,18,22} The most common ABHD5 mutation was found in our patients in homozygous state. It consists of an insertion of a cytosine in position 594 (c.594insC) (Figure 1c). The addition of this supplementary base should lead to the production of a truncated protein (p.N209*). However, it is likely that the mRNA containing the premature stop codon is going to be degraded through the nonsense-mediated mRNA decay (NMD) mechanism. Indeed, thanks to NMD the cells survey mRNA quality and are able to eliminate those mRNAs carrying premature termination triplets, preventing the synthesis of truncated proteins.\textsuperscript{23} In CDS patients the lack of ABHD5 protein determines a lower efficiency of ATGL activity. ATGL catalyzes the first step in the hydrolysis of TAGs generating diacylglycerols (DAGs) and free fatty acids (FAs), stored into cytoplasmic lipid droplets (LDs). DAG is the major substrate for hormone-sensitive lipase (HSL) activity, which determines the production of FA and

\begin{figure}[h]
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\caption{Clinical and microscopic findings of patient 1. (a) and (b) Microscopic view of granulocytes stained with May-Grünwald Giemsa showing Jordan’s anomaly (arrows). Congenital ichthyosis on trunk and limbs before (c, black circle, and d) and after acitretin treatment (e, black circle, and f). The treatment ameliorated general skin conditions.}
\end{figure}

\begin{figure}[h]
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\caption{Clinical, microscopic and radiological findings of patient 2. Lamellar ichthyosis, shown by arrows, affecting facial region (a), trunk (b) and extremities (c-e). Jordan’s anomaly, indicated by arrows, in May-Grünwald Giemsa-stained buffy coats (f-h). Periodontitis (i) and periodontal recession displayed by panoramic radiograph (j).}
\end{figure}
MAG (monoacylglycerol). Finally, monoglyceride lipase (MGL) catabolizes MAG to FA and glycerol. FA generated from these processes are major energy substrates for cell. In CDS patients, the biochemical pathways reported above are largely compromised, because of ABHD5 molecular defect. Consequently, a systemic increase in the size and number of LDs, and a decrease of FA availability can be detected (Figure 4). In many articles, a beneficial effect on CDS patients following a low triglyceride diet supplemented with MCT oil has been reported. This is probably due both to a decrease accumulation of TAGs into LDs and an increase the availability of energy by the oxidation of MAG (monoacylglycerol).
medium chain FAs. Total or partial loss of ABHD5 function causes systemic manifestations, including ichthyosis, liver, muscle and central nervous system injury and elevation of hepatic and muscle enzymes. Ichthyosis is present in 100% of patients, while liver involvement can be detected in 85% of cases.\(^1,12\) Accordingly, our patients show NCIE, which improved only after three months of acitretin treatment. They presented also hepatic steatosis and elevated levels of liver enzymes, therefore a specific diet was recommended. Moreover, in our patients, signs of myopathy were detected by electromyography and were associated to elevated levels of CK. ABHD5 gene analysis was performed in two thirds of CDS patients (103/151). Among them, 83 subjects harbored severe mutations (causing truncated protein or no protein production), while the remaining 20 presented mild mutations (missense mutations). In the first group of patients (severe mutations, including the p.N209* mutation), signs of myopathy and elevated level of muscles enzymes (CK or CPK) were detected in 36% and 67.5% of cases, respectively. Moreover, electromyographic study revealed early recruitment with complete interphase pattern suggestive of myopathic pattern. Instead, in the second group of patients (missense mutations), myopathy and elevated level of muscles enzymes were observed in 15% and 30% of cases, respectively (Figure 5). The evaluation of myopathy and CK levels displayed significant difference between the two groups of patients, thus indicating that total or partial impairment of ABHD5 function causes a variable muscle damage, depending from the different availability of energy. As muscles require a lot of energy to work, the partial or total impairment of ABHD5 ability to activate ATGL hinders chemical reactions involved in TAG hydrolysis, determining their build-up inside muscle cells and energy levels too low. A skeletal muscle myopathy may occur, especially in the cases of total lack of ABHD5 protein. Considering patients with myopathy, during successive decades of life (total: 33 patients), we can observe that the levels of CK and CPK increased similarly (2-3 fold) in all the intervals of times and that the myopathy, detected mainly by EMG examination, is mild or very mild in all cases and over time (Table 1).

In conclusion, we described the clinical and genetic findings of two unreported CDS patients from different Turkish families. As CDS is an extremely rare genetic disorder and its pathophysiology is largely unknown, it is important to expand the cohort with novel patients and evaluate genotype-phenotype correlations.

**List of acronyms**
ABHD5 - α/β-hydrolase domain-containing protein 5; ATGL - adipose triglyceride lipase; CDS - Chanarin-Dorfman Syndrome; CK - creatine kinase; DAG - diacylglycerol; EMG - electromyography; FA - fatty acid; HSL - hormone-sensitive lipase; LD - lipid droplet; MAG - monoacylglycerol;
MGL - monoglyceride lipase
NCIE - non-bullous congenital ichthyosiform erythroderma;
NLSDI - Neutral Lipid Storage Disease with ichthyosis;
NLSDM - Neutral Lipid Storage Disease with myopathy;
NMD - nonsense-mediated mRNA decay;
TAG - triacylglycerol

Authors contributions
DT wrote and edited the manuscript; MD and CA performed clinical evaluation; ET carried out molecular analysis; SM conceived the study and supervised it. All authors read and approved the final manuscript.

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Conflict of Interest
The authors declare they have no financial, personal, or other conflicts of interest.

Ethical Publication Statement
We confirm that we have read the Journal’s position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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