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RAPID COMMUNICATION

A novel *ABHD5* mutation in two Chanarin Dorfman siblings with severe and heterogeneous clinical phenotype

Chanarin Dorfman Syndrome (CDS; MIM: 275630) is a rare autosomal recessive disorder, characterized by triacylglycerol (TG) accumulation in lipid droplets (LDs) within different tissues including skin, liver, skeletal muscle, bone marrow, eyes, ears, and central nervous system.[1] In CDS, the prevalent and always observed clinical feature is a non-bullous congenital ichthyosiform erythroderma (NCIE). Liver and muscle involvement is detected in more than 80% and 40% of cases, respectively. Neurological symptoms have been reported in almost 30% of affected subjects. The disease is due to mutations in the α/β-hydrolase domain-containing protein 5 (ABHD5). This protein can activate both adipose triglyceride lipase (ATGL), the first rate-setting enzyme in TG hydrolysis, and PNPLA1, which catalyzes the final step of ω—O-acylceramide production (Fig. S1A, B).[2]

Until now, 45 different ABHD5 mutations have been reported in CDS patients. Most of them (77%) cause the generation of truncated proteins, while the remaining ones are missense mutations that could partially reduce ABHD5 activity (Fig. S1C).[1]

Here we describe a novel homozygous 13 bp deletion, within exon 4 (Fig. 1A), (c.553delTTGGGGTTTCCCT, GenBank accession number OL860961) of *ABHD5* gene in two young siblings affected by CDS, a 12.5-year-old boy (Patient 1) and a 20-month-old boy (Patient 2), sons of first cousins. Parents were identified as carriers (Fig. 1A). The 13 bp deletion resulted in a frameshift mutation that may lead to a shorter ABHD5 protein (p.W179Nfs22*), lacking of those amino acids involved in ATGL activation (Fig. S2).

Bioinformatic analysis performed by I-Tasser software revealed alterations of ABHD5(W179Nfs22*) secondary structure. In particular, a modification of two residues, S69 and G87, within the hydrophobic domain, was predicted. Changes involving amino acids of this domain could abolish the binding of ABDH5 protein to LD, totally abrogating protein function. An incorrect tridimensional folding was also predicted for the truncated mutant protein (Fig. 1B, C).

Although both patients carried the same ABHD5 mutation and grew up in the same family, clinical heterogeneity has been observed and seems to suggest the influence of other factors, which could modulate the severity of the systemic damage.

In Patient 1 was noticed a generalized ichthyosis since birth. At the age of 8 months, parents also noticed abdominal distension and investigations revealed an increase in liver enzymes. At the age of 18 months, he was referred to Yassin Abdel Ghaffar Center for liver disease and research with hepatomegaly and developmental delay. He was able to sit unsupported but was unable to stand or walk without support. His liver was firm and enlarged 12 cm below costal margin in the midclavicular line with rounded border and smooth surface. Spleen was felt 3 cm below the costal margin. A provisional diagnosis of CDS was made and confirmed by the presence of vacuolated granulocytes (Fig. 1D). Liver biopsy (at the age of 3 years) showed diffuse marked macro-vesicular steatosis in hepatocytes and a mononuclear inflammatory cellular infiltrate associated with mild fibrosis into the portal areas (Fig. S3A, B). Although liver involvement is reported in most of CDS subjects, only half of them develop both hepatomegaly and steatosis or cirrhosis and less than half show also hepatic fibrosis. These findings indicate that the severity of liver symptoms is highly variable in CDS.[3] Patients with severe liver damage usually present mutations deeply affecting the structure of ABHD5 protein or causing the absence of protein production, as in the case of our patient. Nevertheless, many studies highlighted the complexity to establish a clear genotype—phenotype correlation.[4] During the last examination at the age of 12.5 years, NCIE was still present in our patient (Fig. S3C, D). He had average mentality and was at grade 7 in normal stream school. His liver was still enlarged 8 cm below the costal margin, firm with rounded border and smooth surface. Abdominal US confirmed hepatomegaly and splenomegaly with no signs of portal hypertension. Fibroscan analysis

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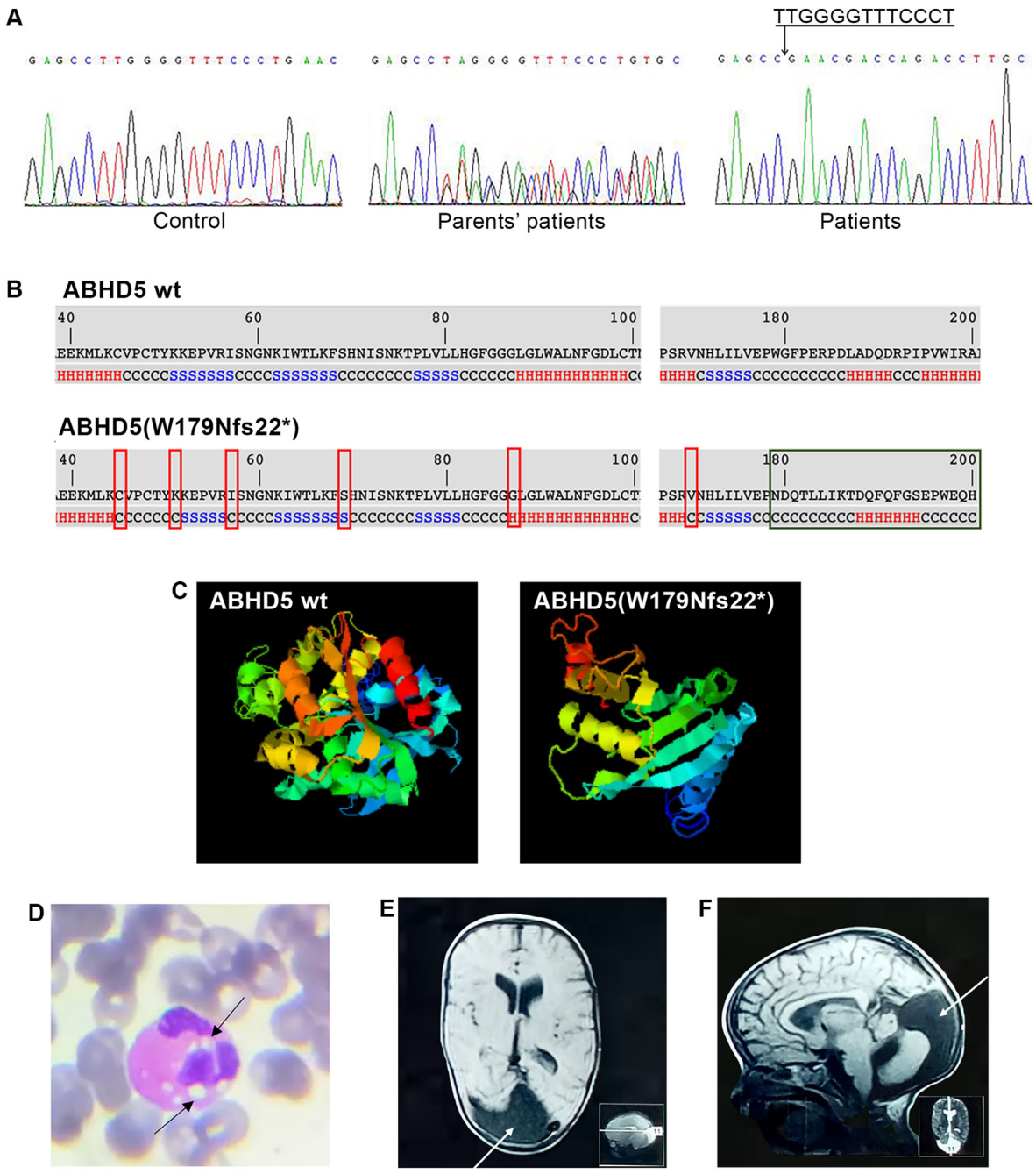

Figure 1 Molecular and clinical features of patients 1 and 2. **(A)** Electropherogram of *ABHD5* exon 4 sequence harboring the c.553delTTGGGGTTTCCCT mutation in homozygous status in patients and in heterozygous status in parents, compared to a control sequence. **(B)** Secondary structure of ABHD5 (W179Nfs22*) compared to wild type ABHD5 protein. Deletion causes some modifications of the protein structure at position 45 (from α-helix to coiled-coil), 51 (from β-sheet to coiled-coil), 57 (from β-sheet to coiled-coil), 69 (from coiled-coil to β-sheet), 87 (from coiled-coil to α-helix), and 170 (from coiled-coil to α-helix). The changes are marked by red rectangles. Mutant amino acid sequence, due to frameshift mutation, is indicated by green rectangle. **(C)** Predicted 3D structure of ABHD5 and ABHD5 (W179Nfs22*) proteins. The p.W179Nfs22* mutation causes dramatic conformational modifications affecting protein folding. **(D)** Microphotographs of May-Grünwald-Giemsa buffy coats showing lipid-containing vacuoles (arrows). **(E, F)** Sagittal and coronal sections show cerebellar hypoplasia and asymmetrical dilation of lateral ventricles in the brain (arrows). There is enlargement of the posterior fossa containing cerebrospinal fluid and upward displacement of the tentorium.

showed stiffness of 12.9 Kpascal (stage of fibrosis F3). A mild elevation in liver enzymes was noticed through 10 years follow up (ALT: 46—78 U/L ($N = 5$—35); AST: 50—97 U/L (N up to 40)). Complete blood count revealed a mild anaemia with normal WBCs, and a decrease in platelets. EMG and NCV showed no abnormalities. The patient presented a decrease in bone density below the normal for the same age and sex group measuring −1.8 of the Z score, and normal bone mineral density of the total body. Audiometry revealed bilateral conductive hearing loss. Chest and heart were clinically free.

Patient 2 had tachypnea soon after birth and was admitted to the Neonatal Intensive Care Unit for 20 days, during which he received only oxygen by mask. Generalized ichthyosis was noticed at birth like his older brother. At the age of 2 months, brain MRI revealed hydrocephalic changes and cerebellar hypoplasia (Fig. 1E, F). Neurosurgery consultation advised a shunt operation which was done at the age of 5 months. After 7 months, the increase in liver enzymes was noticed during routine work up and the patient was referred to Yassin Abdel Ghaffar Center for liver disease and research. On examination, his length was 73 cm (−1.16 SD), weight was 7 kg (−2.9 SD), skull circumference was 46 cm (−0.05 SD). He had severe global developmental delay and he was not able to support his head or follow objects. Abdominal US showed mild hepatomegaly. His liver was 5 cm below the costal margin in the midclavicular line, firm, with a rounded border, and a smooth surface. Spleen was not felt. Chest and heart were clinically free. EEG and echocardiography were normal. He had generalized severe hypotonia and increase in deep tendon reflexes. Hearing loss was not observed. A mild-moderate elevation in ALT and AST liver enzymes (ALT: 45 U/L; AST: 40 U/L) and mild anemia were detected. The patient died at 2 years of age with pneumonia complications. Severe global developmental delay, Dandy Walker malformation and cerebellar hypoplasia are unusual manifestations in CDS patients. Cerebellar malformation could result from a combination of genetic and environmental factors that affect development before birth, or it could be associated with chromosomal abnormalities. Normal karyotype identified in patient 2 allows to exclude a chromosomal aberration as the cause of cerebellum hypoplasia. Different neurological dysfunctions have been reported in CDS patients (Table S1). Moreover, brain MRI/MRS and spectroscopy analysis also revealed abnormal lipid accumulation in cerebral white matter, cortex, and basal ganglia of some CDS patients.[5] Although no ATGL mutations are present in CDS, lipase activity is impaired by ABHD5 mutations, causing the decrease of TG metabolism and the abnormal storage of neutral lipids in the brain, as well as in the other tissues. To date, the molecular mechanisms underlying the accumulation of neutral lipids in CDS neurons are completely lacking.

Here, we describe a novel *ABHD5* frameshift mutation, associated with a severe manifestation of CDS. We report a severe multisystemic involvement in two patients, and an unusual neurological manifestation in one of them. In CDS, as well as in other genetic disorders, the genotype—phenotype correlation cannot completely explain clinical variability. For this reason, further investigations, involving CDS patients from different countries, should be performed to evaluate the possible role of lifestyle, epigenetic mechanisms or variation in any putative modifier gene expression.

Abbreviations

ABHD5	α/β-hydrolase domain-containing protein 5
ALT	alanine aminotransferase
AST	aspartate aminotransferase
ATGL	adipose triglyceride lipase
CDS	Chanarin Dorfman syndrome
CPK	creatine phosphokinase
EEG	electroencephalography
EMG	electromyography
FA	fatty acid
LD	lipid droplet
MGG	May-Grünwald-Giemsa
MRI	magnetic resonance imaging
NCIE	non-bullous congenital ichthyosiform erythroderma
NVC	nerves conduction velocity
TG	triacylglycerol
US	ultrasound

Ethics declaration

Ethical approval and informed consents were obtained from the study participants.

Author contributions

SME was responsible for patient care; TYA, KZ and E performed clinical investigations; genetic analysis was performed by SM; bioinformatic analysis was carried out by ET and LM; detailed clinical data and materials for molecular studies were provided by SME; evaluation of neurological diagnosis was performed by CA; the paper was written by SME, SM and DT; the study was designed and coordinated by DT. All authors read, critically revised and approved the final manuscript.

Conflict of interests

Authors declare no conflict of interests.

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Availability of data and materials

Data obtained during this study are included in the article.

Consent for publication

Written informed consents were obtained from parents of patients for publication of this article and any accompanying images.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gendis.2022.08.005.

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