Short Communication

A novel nonsense *ATP7A* pathogenic variant in a family exhibiting a variable occipital horn syndrome phenotype

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A R T I C L E I N F O

A B S T R A C T

We report on a family with occipital horn syndrome (OHS) diagnosed in the proband's late fifties. A novel *ATP7A* pathogenic variant (c.4222A > T, p.(Lys1408*)), representing the first nonsense variant and the second late truncation causing OHS rather than classic Menkes disease, was found to segregate in the family. The predicted maintenance of transmembrane domains is consistent with a residual protein activity, which may explain the mild clinical presentation.

1. Introduction

Occipital horn syndrome (OHS, OMIM #304150) takes its name from the pathognomonic occipital horn exostoses. It presents in early to middle childhood and is characterized by connective tissue and skeletal manifestations [1,2]. Dysautonomia and subtle cognitive deficits might be displayed by some OHS patients; neurological manifestations are predominant in the allelic classic Menkes disease (MD, OMIM #309400). Both disorders, which represent the ends of a continuum, display X-linked recessive inheritance and are caused by pathogenic variants in the *ATP7A* gene, which codes for a transmembrane copper-transporting ATPase that cycles between the trans-Golgi (delivery of copper to the secreted copper enzymes) and the plasma (export of surplus intracellular copper) membranes. The phenotypes are mainly related to a deficient activity of cuproenzymes [2,3]. OHS and mild MD are usually the consequences of pathogenic variants that would result in a protein with residual activity, such as splice-site variants which also produce small amounts of normal protein [4–10]. Truncating variants, such as exon deletions or nonsense variants, result instead in MD severe

classical form with death in early childhood [7,8]. In addition, a small number of *ATP7A* missense variants has been recently shown to cause adult-onset isolated distal motor neuropathy, which represents a third distinct phenotype. This neuropathy is mainly caused by subtle defects in ATP7A intracellular trafficking, resulting in preferential localization of the protein at the plasma membrane [11]. We describe a family with OHS first diagnosed in the proband's late fifties. A novel nonsense pathogenic variant in the *ATP7A* gene was found to segregate in the family.

2. Materials and methods

2.1. Clinical report

The patient's medical history started at the age of 5 years (Fig. 1A), when he required bladder catheterisation, due to bladder diverticula. At the age of 47 years he had a 20 cm decrement in height with a forward spine curvature (Fig. 1B, C), causing shortening of breath, feeding difficulties, worsening of walking, and urinary disturbances. Free of
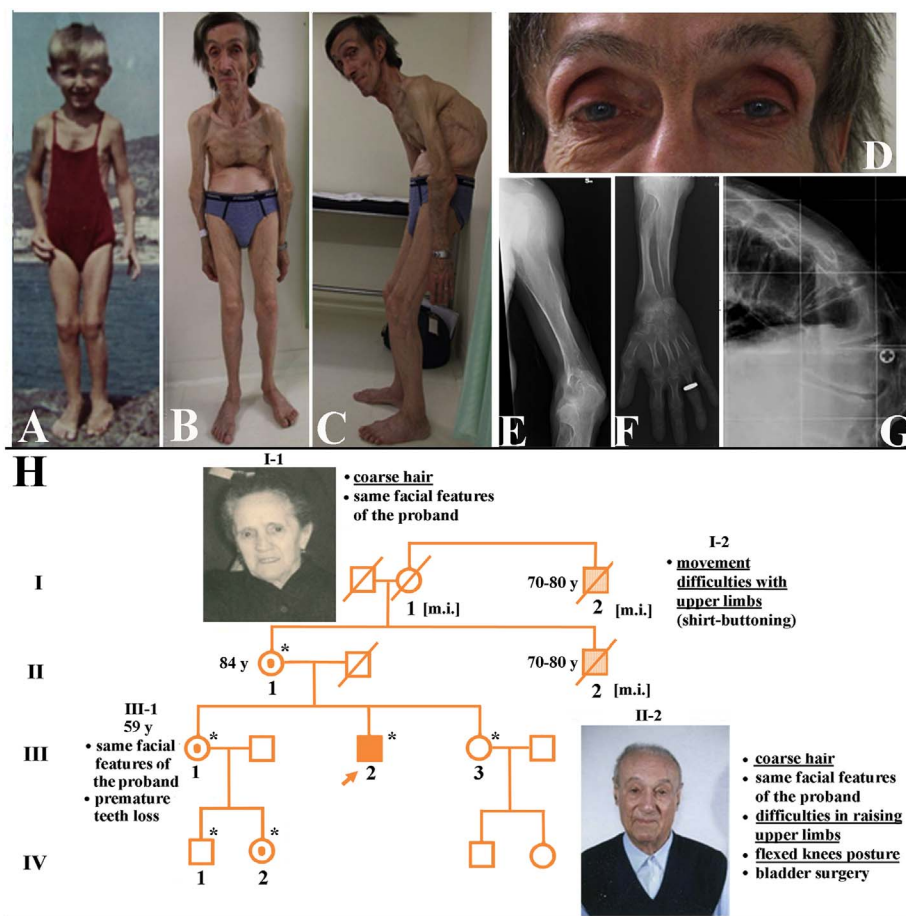
Fig. 1. Proband's evolution of the phenotype, his pedigree and family history, and genetic data. A. Photograph of the proband at the age of 4–5 years, documenting that pectus excavatum, flexion of elbows and knees were already manifested. He used to wear a female swimming suit to cover pectus excavatum. B, C. Long thin face, high forehead, hooked nose, short clavicles, severe angled kyphosis, bony outgrowths at the elbows, absence of subcutaneous fat and reduction of muscle mass represent the current phenotype. D. Detail of the proband's face showing prominent orbital arches, iris depigmentation and coarse hair. E–G. Proband's radiographs of the left elbow, forearm and column, showing: (E–G) diffuse severe osteoporosis; (E, F) the exostoses at the head of ulna and radius and its dislocation; (F) metaphyseal flaring and dyaphiseal wavy contours of long bones; (G) wedge-shaped D11 vertebrae, representing the fulcrum of his severe-angled kyphosis, and L1 vertebrae. H. Filled symbol and symbols filled in lighter color represent, respectively, the proband and his two possibly more mildly affected male relatives (see the details in the clinical report). Female carriers of the pathogenic variant are shown with a dot inside the symbol; asterisk, members genotyped in the study; [m.i.], pathogenic variant inferred; y, years. I. From top to bottom: electropherograms of the *ATP7A* gene sequence of wild type (III-3), carriers (II-1, III-1, and IV-2), and the proband (III-2); schematic view of ATP7A cDNA and protein structure showing in red the approximative position of the pathogenic variant (modified from [24]). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
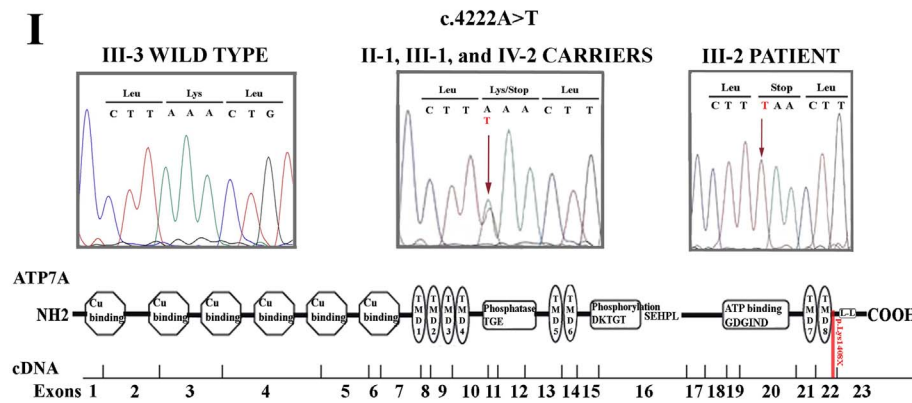
catheterisations from the age of 10 years until that of 20, he suffered from neurogenic bladder requiring three to four catheterisations a day. As a heavy smoker, he also had chronic obstructive pulmonary disease. Colonic diverticula were diagnosed at the age of 52. Premature teeth loss and surgery for a left inguinal hernia complete his clinical history. Physical examination at 57 years showed the features displayed in Fig. 1B–D, as well as limited extension at the elbows, loose palmar skin, hyperextensible interphalangeal joints of the hands, and dystrophic nails. Head circumference was 57 cm (90th p), height 150 cm.

Radiographs of the skeleton demonstrated generalized severe osteoporosis (Fig. 1E–G), that contrasted with the occipital horn exostoses (Supplementary Fig. S1A) and the hyperostosis of the proximal ulna and radius (Fig. 1E, F). Glenohumeral joint alteration (Fig. S1C), together with focal hyperostosis of the elbows and dislocation of the head of the radius into the olecranon (Fig. 1E, F), may explain the patient's inability to touch his head with his hands and the weak grip, which have been

present since childhood. The finding of an open anterior fontanel fitted with a sensation of brain splitting (Fig. S1B). Neuroimaging was precluded by his inability to enter scanners. The family pedigree is shown in Fig. 1H.

Informed consent was obtained from all participants; the study was approved by the Research Ethics Committee of IRCCS Istituto Auxologico Italiano.

2.2. Molecular analysis

ATP7A exons and intron–exon junctions (GenBank accession no. **NM_000052.6**, GRCh37/hg19) were analysed by Sanger sequencing in the proband's DNA extracted from peripheral blood. Available relatives were genotyped using exon 22-targeted primers. Variant *in silico* analysis was performed by consulting: Ensembl Genome Browser [12], Single Nucleotide Polymorphism database (dbSNP) [13], and Leiden

Open Variation Database (LOVD) *ATP7A* [14].

3. Results

Blood chemistries demonstrated in the proband low serum levels of copper (40 μg/dL, reference 70–140) and ceruloplasmin (12 mg/dL, reference 20–60). *ATP7A* sequencing revealed a novel nonsense pathogenic variant in exon 22 (c.4222A > T, p.(Lys1408*)) (Fig. 1I), which was never reported in either healthy subjects or MD/OHS patients. The patient's mother (II-1), the eldest of his two sisters (III-1), and her daughter (IV-2) were found to carry the pathogenic variant (Fig. 1H, I).

4. Discussion

We have described a cognitively normal OHS male, being much older than most OHS or mild MD patients reported in literature [3,14]. The most disabling features were provoked by severe osteoporosis. Inheritance of the pathogenic variant from the patient's mother (Fig. 1H, II-1) suggests that his maternal uncle (II-2) and granduncle (I-2) could also be affected by OHS. Indeed, they both experienced limited movements of shoulders/upper limbs that suggest the presence of the typical OHS skeletal findings described in the proband (Fig. S1). Moreover, the reported presence of coarse hair in the maternal uncle (II-2) and grandmother (I-1) [10], a possible obligate carrier of the pathogenic variant, further supports this hypothesis.

All the reported *ATP7A* truncating variants, either early or late [8,14,15], cause the classic/severe-classic form of MD, except for the pathogenic variant described by Dagenais et al. [16] and that reported here. Specifically, the OHS family described by Dagenais et al. [16] was found to carry a frameshift variant (c.4352delG) at codon 1451 in exon 23, which gave rise to 13 novel amino acids before a premature stop codon. Apart from the low-average range IQ, the proband's phenotype seems to overlap that of the present patient. In addition, as a result of late truncating variants, both *ATP7A* transcripts are predicted to lack the distal di–leucine motif, which is considered essential for the protein recycling between the trans-Golgi and the cell membranes. Accordingly, Dagenais et al. [16] demonstrated in their patient abundant *ATP7A* transcript levels but reduced levels of the corresponding truncated protein, which they proposed to be predominantly located in the plasma membrane. The phenotype was considered as consistent with lysyl oxidase (LOX) – which is involved in collagen and elastin cross-linking – being more sensitive than other cuproenzymes to copper deficiency [16], as LOX needs copper during its synthesis in the endoplasmic reticulum.

Furthermore, by restricting the review to *ATP7A* truncating variants affecting either exon 22 or 23 [7–8,14,17–20], the present novel pathogenic variant – as well as that of Dagenais et al. [16] – differs from the other truncations as it is predicted to preserve the eighth transmembrane domain (TMD8), introducing a stop codon just downstream of the predicted TMD8 (Fig. 1I). Conversely, a frameshift variant at position 1400, just 8 codons before the variant here described, but affecting the TMD8, causes a classic MD [20]. This is consistent with the fact that the structure of ATP7A transmembrane domains are extremely conserved and even mild alterations are known to lead to marked reductions in protein activity [8].

5. Conclusion

To our knowledge, the present report is the first to display a nonsense variation resulting in an *ATP7A* late truncation associated with OHS rather than MD. The patient's phenotype suggests that ATP7A retains a certain degree of residual activity when TMD8 remains intact, despite the absence of the C-terminal 93 amino acids. The limit of truncating variants with residual enzymatic activity can therefore be shifted from exon 23 (codon 1451) to exon 22 (codon 1408). Moreover,

the patient's possibly affected male relatives further support the idea that the intrafamilial phenotypic variability might be very wide when the variant has a residual enzymatic activity [4,21–23], and may be due to different expression of the mutant *ATP7A*, as previously demonstrated [23]. As a consequence of OHS phenotypic variability, males with ID and connective tissue abnormalities should be evaluated for biochemical evidence of defective copper transport.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ymgmr.2017.07.007.

Conflict of interests

None to declare.

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