Ataxia with Ocular Apraxia Type 1 (AOA1) (*APTX*, W279* Mutation): Neurological, Neuropsychological, and Molecular Outlining of a Heterogenous Phenotype in Four Colombian Siblings

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Abstract

Hereditary ataxias are a group of devastating neurological disorders that affect coordination of gait and are often associated with poor coordination of hands, speech, and eye movements. Ataxia with Ocular Apraxia type 1 (AOA1) (OMIM: 606350.0006) is characterized by slowly progressive symptoms of childhood-onset and pathogenic mutations in *APTX;* the only known cause underpinning AOA1. *APTX* encodes the protein Aprataxin, composed of three domains sharing homology with proteins involved in DNA damage, signaling, and repair. We present four siblings from an endogamic family in a rural, isolated town of Colombia with ataxia and ocular apraxia of childhood-onset and confirmed molecular diagnosis of AOA1, homozygous for the W279* p.Trp279Ter mutation. We predicted the mutated APTX with Alpha Fold to demonstrate the effects of this stop-gain mutation that deletes three beta helices encoded by amino acid 270 to 339 rescinding the C2H2-type zinc fingers (Znf) (C2H2 Znf) DNA-binding and DNA-repair domain and the whole tridimensional structure of the APTX. All siblings exhibited different ages of onset (4, 6, 8, and 11 y/o) and heterogeneous patterns of dysarthria (ranging from absence to mild-moderate dysarthria). Neuropsychological evaluation showed no neurocognitive impairment in three siblings, but one sibling showed temporospatial disorientation, semantic and phonologic fluency impairment, episodic memory affection, constructional apraxia, moderate anomia, low executive function, and symptoms of depression. This heterogeneous phenotype suggests genetic interactions can shape the natural history of AOA1. To our knowledge, this report represents the most extensive series of siblings affected with AOA1 in Latin America, and the genetic analysis completed adds important knowledge to outline this family's disease and general complex phenotype of hereditary ataxias.

Introduction

Hereditary ataxias are a group of genetic disorders which affect the coordination of gait and are frequently associated with poor coordination of hands, speech, and eye movements. The hereditary ataxias can be subdivided first by their inheritance (i.e., autosomal dominant, autosomal recessive, X-linked, and mitochondrial) and secondarily by the gene in which pathogenic variants occur or the chromosome locus to which the phenotype has been mapped.[1–3]

Most autosomal recessive ataxias are of early onset and share spinocerebellar ataxia as the critical feature, involving the cerebellum, brainstem, or long spinocerebellar tracts.[4–6] In contrast to the autosomal dominant ataxias, autosomal recessive ataxias are generally associated with peripheral sensorimotor neuropathy and have an effect outside the nervous system.[4–8] Acquired causes of ataxia such as alcoholism, vitamin deficiencies, multiple sclerosis, vascular disease, meningitis, tumors, paraneoplastic diseases, viral cerebellitis, and cerebellar abscess need to be considered in everyone with ataxia in search of treating the cause with specific treatment.[9–11]

Ataxia and Oculomotor Apraxia type 1 (AOA1) is a specific type of hereditary ataxia found in patients with mutations in the *APTX* gene and a clinical presentation accompanied by oculomotor apraxia.[1,12–16] The *APTX* gene encodes for a protein called Aprataxin, composed of three domains that share distant

homology with the amino-terminal domain of polynucleotide kinase 3'- phosphatase (PNKP), with histidine-triad (HIT) proteins and with DNA-binding C2H2 zinc-finger proteins.[14,15,17–22] Given the role of PNKP in DNA single-strand break reparation, Aprataxin has been involved in the repair of DNA in cells of various tissues, including the brain, spinal cord, and muscles.[15,23–26] The APTX gene is widely expressed in the nervous system and detected in the cerebellum, basal ganglia, cerebral cortex, and spinal cord.[23,25] Over 30 mutations in the APTX gene have been described to cause AOA1. In autopsied cases, cerebellar atrophy has been associated with a severe loss in Purkinje cells and degeneration of posterior columns, spinocerebellar tracts, and spinal cord anterior horn cells.[5,6,11,13,14,17] In a recent study, the nonsense mutation W279*, first reported in association with a Portuguese founder haplotype, was the most frequent mutation, representing 66% of all mutated alleles.[27]

During the period of the clinical evolution of AOA1, the first disease symptoms correlate with poor coordination and ataxia.[15,27] The onset-age of symptoms is ~4 y/o (range 2-10 y/o). Along with ataxia and oculomotor apraxia, affected individuals can show dysarthria, chorea, myoclonus, and cognitive defects.[15,27] neuropathy affects almost every patient and leads to impaired reflexes, limb weakness, and pallesthesia deficits.[15,27] This neurological compromise affects self-ambulatory displacement requiring external assistance ten to15 years after the onset of the first symptoms.[1,3,4,8] Ocular motor apraxia is a prominent clinical feature necessary for being able to characterize patients with AOA1. The oculomotor apraxia pattern of this disease is characterized by increased latencies and decreased amplitude of horizontal saccades while vertical saccades are reportedly normal, a description compatible with the apraxia in our patients.[1,3,4,8,15,27]

This article describes the case of a consanguineous pedigree with twelve siblings, four of them with ataxia and ocular apraxia symptoms of childhood-onset, inhabiting a rural, isolated, and poor community from Colombia, in which the molecular assessment with next-generation sequencing allowed us to define the clinical diagnostic of AOA1 after characterizing an APTX mutation. We present in detail the neurological and neurocognitive progression of the disease, the molecular characterization of the W279* p.Trp279Ter mutation (NC_000009.11:g.32974493C>T), the comprehensive application of bioinformatic analyses directed to define the structural compromise of the mutated protein and the interaction with other molecules.

Patients And Methods

In May of 2017, a commission composed of two neurologists (DA, DH) and two neuropsychologists (SM, LM) traveled to the municipality of “Ariguaní” (town of “El Difícil”) in the State of Magdalena, Colombia (Figure 1a) where the affected individuals live. The family's home is far away from the populated area, and there is no road available for motorized vehicles (Figure 1a). Therefore, the trip must be completed by horse or by foot. Once in place, the reconstruction of the pedigree was done by anamnesis following standard procedures. The nuclear family, including the four symptomatic individuals, derives from the mating of two consanguineous first cousins. Endogamous marriage is a common phenomenon in this

geographical region because emigration and immigration are limited by the geographical barriers and because the territory is plagued of guerrillas, paramilitary groups and narcotics smugglers. Both parents are unaffected, and the four affected individuals are from a sibship of 12 sibs (Figure 1b). There are twelve descendants in the last generation of the pedigree (Figure 1b).

Neurological and neuropsychological assessment: The anamnesis of symptoms and signs as well as the neurologic examination and other clinical procedures were applied as standardized by the Grupo de Neurociencias de Antioquia form the Medical School, Universidad de Antioquia, Medellín, Colombia and fully described elsewhere[28–31] and in the web page: https://web.gna.org.co/en/clinical-studies/

The protocol used for neuropsychological assessment comprises the battery of the CERAD: Mini-Mental State Examination, Semantic Fluency (Animals), CERAD Boston Naming Test, CERAD Word List Learning and delayed recall, CERAD Constructional Praxis Copy and delayed recall.[32–34] Episodic memory tests were applied: MIS - Memory Impairment Screening and MCT - Memory Capacity Test.[35,36] It is part of the protocol the Rey-Osterrieth Complex Figure Copy and, Phonemic Fluency (FAS) “F”, Wisconsin Card Sorting Test, Trail Making Test -Part A WAIS-III Digit Symbol, Raven’s Progressive Matrices A. The Functional Scales: Barthel, EDG, Katz, Lawton-Brody, Yesavage - Depression Scale, and Memory Disorders Scale, patient, and caregiver. These methods have been described in detail elsewhere.[28–31]

Case 1

The index case is the older sibling, a 38-year-old illiterate man; his psychomotor development was normal until 5. He debuted with a slow gait with lateral deviation and instability, frequent falls, paresthesias, and occasional finger pain compromise of muscle strength. At 35, he showed calm behavior, regular sleep, and a good appetite and denied memory loss, delusions, hallucinations, disinhibition, depressive episodes, seizures, myoclonus, emotional incontinence, urinary and fecal incontinence. Nevertheless, liquids dysphagia, steppage gait, bilateral foot drop were noticed. The man required assistance for daily life activities. Physical examination showed an alert and collaborative person, facial dyskinesia, mild to moderate dysarthria and intelligible speech, horizontal ocular apraxia, and dysmetria. Both upper and lower extremities showed distal muscle atrophy without pes cavus; muscle strength was 4/5; upper and lower extremities exhibit hyporeflexia and Achillean areflexia, distal hypotonia was also identified, neuropathic gait, and bilateral foot drop. Hoffman, Trommer, and Babinski’s signs were negative. In the upper extremities, a glove spreading hypoalgesia; in lower extremities, bilateral hypoalgesia, starting al L1, were detected (a summary of neurological signs and symptoms for each patient is presented in Table 1). The neuropsychological assessment suggests time-spatial disorientation with impaired cognitive domains and difficulty for complex daily-life activities due to motor symptoms. There were no symptoms or signs of dementia (Table 2).

Table 1
Demographic, epidemiologic, neurological signs, and symptoms differences and similarities found in the clinical examination of the 4 siblings affected with Ataxia with Ocular Apraxia type 1 (AOA1) (*APTX*, W279*, p.Trp279Ter mutation).

Sign and Symptoms	Case 1	Case 2	Case 3	Case 4
Complications during pregnancy birth and birth	No	No	No	No
Age of onset (years)	5	6	11	8
Instability and frequent falls	Yes	Yes	Yes	Yes
Paresthesias	Yes	No	No	No
Physical pain	Occasional	No	No	No
Dysphagia	Yes	No	No	No
Behaviour	Calm	Irritable	Calm	Aggressive
Sleeping patterns and appetite	Normal	Normal	Normal	Normal
Urinary and fecal incontinence	No	No	No	No
Memory	Normal	Normal	Normal	Normal
Delirium, hallucinations or disinhibition	None	None	None	None
Pseudobulbar affect	None	None	None	None
Depressive episodes	No	No	No	No
Seizures and myoclonus	No	No	No	No
Dysarthria	Mild-moderate	Mild-moderate	Mild	N/R
Language	Understandable	Scarce	Understandable	Understandable
Vital Signs	Normal	Normal	Normal	Normal
Facies	Symmetric	Symmetric	Symmetric	Symmetric
Fundus examination	Normal	Normal	Normal	Normal

Sign and Symptoms	Case 1	Case 2	Case 3	Case 4
Telangiectasias	No	No	No	No
Muscle Tone	Lower Limb Hypotonia	Atrophy of Gastrocnemius	Thenar and gastrocnemius hypotrophy and Hypertrophy of both deltoids	Atrophy of Gastrocnemius
Ocular-motor Apraxia	Yes	Yes	Yes	Yes
Strength	4/5	4/5	4/5	4/5
Generalized Hyporeflexia	Yes	Yes	Yes	Yes
Hoffman, Trommer and Babinski Reflexes	Absent	Absent	Absent	Absent
Chorea	No	No	No	No
Sensitivity	Bilateral Glove Hypoalgesia	Conserved	Bilateral Glove-and-stocking Anesthesia	Conserved
Dyskinesia	Upper limb and Facial	Facial	None	Facial
Dysmetria	Yes	Yes	Yes	Yes
Foot Drop	Bilateral	Absent	Bilateral	absent
Cavus Foot	Absent	Absent	Absent	Absent

Table 2
Summary of neuropsychological findings in 4 siblings affected with Ataxia with Ocular Apraxia type 1 (AOA1) (*APTX*, W279*, p.Trp279Ter mutation).

	Case 1	Case 2	Case 3	Case 4
Age	37	30	23	17
Mini-Mental State Examination/30	17	17	15	19
Attention				
Memory				
CERAD Word List Learning /30	11	13	11	17
CERAD Word List Delayed Recall /10	3	4	4	4
CERAD Constructional Praxis Delayed Recall/11	2	0	2	0
Rey-Osterrieth Complex Figure Delayed Recall/36	0.5	0	5.5	0
MIS Memory Impairment Screen Free Recall /8	6	6	6	7
Praxis				
CERAD Constructional Praxis Copy /11	0	0	2	4
Rey-Osterrieth Complex Figure Copy/36	0.5	0	7	22
Language				
CERAD Boston Naming Test /15	6	8	11	8
Semantic Fluency (Animals)	9	5	6	7
Executive Functioning				
Wisconsin Card Sorting Test				
Categories /6	1	N/A	1	1
Perseverations	22	N/A	28	42
Phonemic Fluency (FAS) “F”	1	1	N/A	N/A
Raven’s Progressive Matrices A/12	5	5	8	5
Functional Scales				

Note: Low performance in some neuropsychological tests might be explained by the poor schooling level and there was not available data for:
WAIS-III Digit Symbol
Trail Making Test-A (time)
MCT Memory Capacity Test -Total Free Recall /32

	Case 1	Case 2	Case 3	Case 4
Memory Disorders Scale QP/45 QF/45	27/28	22/30	26/22	22/24
FAST/16	3	3	3	3
EDG/7	3	3	3	3
KATZ/6	2	2	0	0
BARTHEL/50	40	40	50	50
Lawton & Brody/8	3	5	5	3
Yesavage (depression scale)/15	11	10	5	11

Note: Low performance in some neuropsychological tests might be explained by the poor schooling level and there was not available data for:
WAIS-III Digit Symbol
Trail Making Test-A (time)
MCT Memory Capacity Test -Total Free Recall /32

Case 2

Case 2 is the sister of the index case. At age six, she begins to present gait instability and frequent falls. Her menarche was at 12, and she gave birth to a 8-year-old child, born by C-section. At 33-years-old she showed irritable behavior and needed support to develop daily life activities. Physical examination showed steppage and slow gait with lateral deviation, but she was able to walk shortly on flat surfaces without support. Horizontal nystagmus was noticed along with bilateral ocular apraxia, oral dyskinesia, finger-nose dysmetria, mild dysarthria, understandable speech, and low language proficiency. Upper and lower limbs showed distal muscle atrophy, mild atrophy of the gastrocnemius, and muscle strength was 4/5. Brachioradialis hyporeflexia with bilateral bicipital, tricipital, patellar, and Achillean areflexia was noticed. Hoffman, Trommer, and Babinski's signs were negative, and she did not show sensitivity compromise in the limbs (a summary of neurological signs and symptoms for each patient is presented in Table 1). The neuropsychological assessment suggests time-spatial disorientation with low semantic fluency, problems to nominate, constructional praxis and symptoms of depression. She also had impairment for complex and straightforward daily-life activities due to motor symptoms. She did not show signs or symptoms of dementia. **(**Table 2**).**

Case 3

The third affected individual in the family started with difficulty to stand, lateral deviation when walking and frequent falls at eleven years of age. At the age of 26, he showed an ataxic and steppage walking pattern with short steps only in flat sections. He requires assistance with daily living activities and has a mild dysarthria and intelligible speech. Physical examination displays ocular apraxia, horizontal nystagmus, slight dysmetria, pectus excavatum and bradycardia. Hypertrophy of the deltoid muscle was

noticed and hypotrophy of the thenar region and gastrocnemius muscle were recorded. Muscle strength was normal in upper limbs but described as a 4/5 in distal lower limbs. Hyporeflexia/areflexia was recorded in upper and lower extremities and she presented a bilateral foot drop. Hoffman, Trommer, and Babinski's signs were negative. Sensitivity examination showed long superficial glove hypoesthesia and stocking anesthesia (a summary of neurological signs and symptoms for each patient is presented in Table 1). The neuropsychological assessment showed temporospatial disorientation, attention impairment, memory, language, praxis, and executive function. Daily-life activities were impaired due to the motor symptoms but did not have symptoms of dementia (Table 2).

Case 4

The last affected member of this family is a 20-year male, at the time of the interview, with gait instability and frequent falls when walking. This began when he was eight years of age. At the age of 18, difficulty to perform routine life activities and occasional irritable and aggressive episodes were noticed. Physical examination showed that he was able to maintain an unsteady yet unsupported gait. He presented mild dysarthria and understandable language as well as facial dyskinesia without facial muscle weakness. Ocular apraxia, horizontal and vertical nystagmus, mild dysmetria were noticed. The man also had a pectus excavatum. The muscle evaluation showed a normal muscle tone, gastrocnemius muscle atrophy, muscle strength with an overall score of 4/5 in all limbs. His reflexes showed generalized hyporeflexia with Achillean areflexia and normal superficial sensitivity. The neuropsychological assessment showed temporospatial disorientation, semantic and phonologic fluency impairment, episodic memory, constructional apraxia, moderate anomia, and low executive function. He had signs of depression. He was fully independent for complex daily life but did have a multidomain mild cognitive impairment (Table 2).

Whole Exome Capture, Sequencing, And Bioinformatics Analysis

Three methods were applied to DNA quantification, and qualification: (1) DNA purity was checked using the Nanodrop (OD260/280 ratio); (2) DNA degradation and contamination were monitored on 1% agarose gels; (3) DNA concentration was measured using Qubit. DNA samples with OD260/280 ratio between 1.8~2.0 and concentration above 1.0ug were used to prepare sequencing libraries.

Library Preparation for Sequencing

Agilent liquid phase hybridization was applied to enrich whole exons sequenced on an Illumina platform efficiently. Sequencing libraries and capture used Agilent SureSelect Human All ExonV5/V6 (Agilent Technologies, CA, USA) with reagents recommended by the instruction manual and following experimental procedures for optimal results.

Next-Generation Sequencing

Genomic DNA was randomly fragmented to 180-280bp with Covaris cracker, and then DNA fragments were ended polished, A-tailed, and ligated with the full-length adapter for Illumina sequencing. Fragments with specific indexes were hybridized with more than 543,872 biotin-labeled probes after pooling, then magnetic beads with streptomycin were used to capture 334,378 exons from 20,965 genes. After PCR amplification and quality control, libraries were sequenced.

Bioinformatic Analyses

All sequenced data were quality assessed (base quality distribution, nucleotide distribution, and presence of adapters, chimeras, and other contaminants) to identify/remove low-quality data/samples from further analysis. All high-quality data was then be mapped to the human genome assembly using the *bwa-mem* algorithm.[37–39] Aligned files were processed using Genome Analysis Tool Kit (GATK) for base quality recalibration, indel realignments, and duplicate removal.[40–42] This was followed by SNP and INDEL discovery and genotyping (plus phasing where applicable) according to GATK Best Practices recommendations.[40–42] All variant calls were subject to variant quality score recalibration and filtering to remove low-quality variants. The remaining high-quality variants were annotated for predicted functional consequences using the Voting Report Index, including SIFT, PolyPhen2 HVAR, Mutation Taster, Mutation Assessor, FATHMM, and FATHMM MKL Coding. For example, for a conservative filter, simply keep things that have 0, 1, or maybe two tolerated predictions. A more conservative filter would keep based on 3, 4, or 5 Damaging predictions. Many variants do not have five algorithms with non-missing values (see Supplementary Material). Updated annotations from the NCBI, the 1000 genome project, were used to evaluate the novelty and rareness of variants. Methods for the selection of variants have been widely described elsewhere.[42–48]

Tridimensional Protein Reconstruction and Functional Effects by *in silico* Analysis

We use the significant improvement in the accuracy of protein structure prediction recently implemented in AlphaFold[50] that incorporates novel neural network architectures and training procedures based on the evolutionary, physical, and geometric constraints of protein structures. Tridimensional protein structure reconstruction with AlphaFold is vastly more accurate than those obtained by competing methods, *i.e.*, median backbone accuracy, highly accurate side chains reconstruction, accurate domains, and domain-packing prediction, and precise, per-residue estimates of its reliability. Code in Phyton is available upon request.

Results

In summary, all affected siblings presented the first symptoms in their childhood with a progressive course of the disease. In all of them, the gait was unstable for two reasons: the presence of ataxia and neuropathic involvement, especially in lower limbs. Three of them had dyskinesias involving the facial musculature, yet there was no weakness of the bulbar musculature. All of the individuals had dysarthria. There were no signs of optic atrophy in the fundus examination. Eye movements were normal, with the

exception of different intensities of nystagmus amongst three of the patients; one of them presenting alteration in slow visual tracking and another one presenting saccades. All of them had ocular apraxia, a crucial finding for the diagnosis of this disease. All had generalized areflexia except for the styloradial reflex, which was present but with decreased intensity. Additionally, in cases 1 and 3, there was a distal sensory compromise in the four extremities with bilateral foot drop and atrophy of gastrocnemius and distal musculature of both hands, indicating more significant peripheral neuropathy progression in these two siblings. All had dysmetria. None had scoliosis or pes cavus, but interestingly, all three males had pectus excavatum.

The neuropsychological evaluation showed no cognitive impairment in three siblings, but the youngest affected brother showed neurocognitive and psychiatric compromise.

Molecular Genetics And Neurobiological Effects

The capture, sequencing, and bioinformatic analysis of the whole genome exome showed the existence of the pathogenic W279* (p.Trp279Ter, UniProt: Q7Z2E3) mutation, rs104894103 (NC_000009.11:g.32974493C>T), in homozygous state in the four affected brothers *in the APTX* gene. This mutation generates a stop-gain mutation, which results in a premature termination codon (a stop was gained) and probably signals the termination of the translation process of the APTX protein in these patients. Classified by ClinVar as pathogenic (RCV000004681.3), RCV000197775.2). According to TopMed, 41 ‘T’ alleles from 125568 have been characterized (gene frequency of 0.000327, similar to that reported by GnomAD, ALSPAC, and TWINSUK, among others).

Protein analysis of the APTX protein shows three main domains **(**Figure 2a**).** The first domain is located at 20-90 amino acid region and corresponds to the forkhead-associated (FHA) domain, a phosphopeptide recognition domain found in many regulatory proteins **(**Figure 2a**).**[51] The second domain, known as the Histidine Triad (HIT) motif or His-x-His-x-His-x-x (x, a hydrophobic amino acid), is located at the 174-269 domain region and is identified as being highly conserved in a variety of organisms **(**Figure 2a**).**[18]

Finally, the region that we hypothesized that is missing after the stop-gain mutation, at the 270-339 amino acid region **(**Figure 2a**)**, corresponds to the C2H2-type (classical) zinc fingers (Znf) contain a short beta-hairpin and an alpha helix (beta/beta/alpha structure), where a single zinc atom is held in place by Cys(2)His(2) (C2H2) residues in a tetrahedral array. C2H2 Znf’s are the most common DNA-binding motifs found in eukaryotic transcription factors and have also been identified in prokaryotes.[19,52] Transcription factors usually contain several Znf’s (each with a conserved beta/beta/alpha structure) capable of making multiple contacts along with the DNA, where the C2H2 Znf motifs recognize DNA sequences by binding to the major groove of DNA via a short alpha-helix in the Znf, the Znf spanning 3-4 bases of the DNA.[20] C2H2 Znf’s can also bind to RNA and protein targets.[20] Biding properties are characterized by the specific domain amino acid sequence and the linker between the zinc fingers.[20]

Figure 2b shows the schematic view of specific molecules that have been characterized to interact with the APTX protein and its different domains. The description of each interactor is presented in Table 3. The APTX protein, specifically its domain C2H2-type (classical) zinc fingers (Znf), is closely related to DNA binding and repair function. APTX may play a role in single-stranded DNA repair through its nucleotide-binding and diadenosine polyphosphate hydrolase activity.[53–56] Hence, a stop gain mutation causes loss of the C2H2-type (classical) zinc fingers (Znf) domain and probably its DNA repair function. Figure 2c shows the region where the Trp279 mutation occurs, emphasizing the C2H2-type (classical) zinc fingers (Znf) domain interacting with the DNA helix.

Table 3. Interactors with the APTX protein.

Molecular interactor	Molecular Function
APEX1	DNA-(apurinic or apyrimidinic site) lyase; Multifunctional protein that plays a central role in the cellular response to oxidative stress. The two major activities of APEX1 in DNA repair and redox regulation of transcriptional factors.
APLF	Aprataxin and PNK-like factor; Nuclease involved in single-strand and double-strand DNA break repair.
APTX	Aprataxin; DNA-binding protein involved in single-strand DNA break repair, double-strand DNA break repair and base excision repair. Resolves abortive DNA ligation intermediates formed either at base excision sites, or when DNA ligases attempt to repair non-ligatable breaks induced by reactive oxygen species.
ATM	Serine-protein kinase ATM; Serine/threonine protein kinase which activates checkpoint signaling upon double strand breaks (DSBs), apoptosis and genotoxic stresses such as ionizing ultraviolet A light (UVA), thereby acting as a DNA damage sensor.
DCLRE1C	Protein artemis; Required for V(D)J recombination, the process by which exons encoding the antigen-binding domains of immunoglobulins and T-cell receptor proteins are assembled from individual V, (D), and J gene segments.
LIG3	DNA ligase 3; Isoform 3 functions as heterodimer with DNA-repair protein XRCC1 in the nucleus and can correct defective DNA strand- break repair and sister chromatid exchange following treatment with ionizing radiation and alkylating agents.
LIG4	DNA ligase 4; Efficiently joins single-strand breaks in a double- stranded polydeoxynucleotide in an ATP-dependent reaction. Involved in DNA non-homologous end joining (NHEJ) required for double-strand break repair and V(D)J recombination.
MSH6	DNA mismatch repair protein Msh6; Component of the post-replicative DNA mismatch repair system (MMR).
NHEJ1	Non-homologous end-joining factor 1; DNA repair protein involved in DNA nonhomologous end joining (NHEJ) required for double-strand break (DSB) repair and V(D)J recombination.
PARP1	Poly [ADP-ribose] polymerase 1; Involved in the base excision repair (BER) pathway, by catalyzing the poly ADP-ribosylation of a limited number of acceptor proteins involved in chromatin architecture and DNA metabolism.
PNKP	Bifunctional polynucleotide phosphatase/kinase; Plays a crucial role in repairing DNA damage, functioning as part of both the non-homologous end-joining (NHEJ) and base excision repair (BER) pathways.
POLB	DNA polymerase beta; Repair polymerase that plays a key role in base-excision repair.
POLM	DNA-directed DNA/RNA polymerase mu; Gap-filling polymerase repairs DNA double-strand breaks by non-homologous end-joining (NHEJ).
PRKDC	DNA-dependent protein kinase catalytic subunit; Serine/threonine-protein kinase acts as a molecular sensor for DNA damage. She was involved in DNA non-homologous end joining (NHEJ) required for double-strand break (DSB) repair and V(D)J recombination.
TDP1	Tyrosyl-DNA phosphodiesterase. DNA repair enzyme that can remove a variety of covalent adducts from DNA through hydrolysis of a 3'-phosphodiester bond, giving rise to DNA with a free 3' phosphate.

Molecular interactor	Molecular Function
TP53	Cellular tumor antigen p53; Acts as a tumor suppressor in many tumor types; induces growth arrest or apoptosis depending on the physiological circumstances and cell type.
XRCC1	DNA repair protein XRCC1; Involved in DNA single-strand break repair by mediating the assembly of DNA break repair protein complexes.
XRCC4	DNA repair protein XRCC4 involves DNA non-homologous end joining (NHEJ) required for double-strand break repair and V(D)J recombination. Binds to DNA and to DNA ligase IV (LIG4).
XRCC5	X-ray repair cross-complementing protein 5; Single-stranded DNA-dependent ATP-dependent helicase. It has a role in chromosome translocation.
XRCC6	X-ray repair cross-complementing protein 6; Single-stranded DNA-dependent ATP-dependent helicase. It has a role in chromosome translocation.
WRN	Werner syndrome ATP-dependent helicase is a Multifunctional enzyme with magnesium and ATP-dependent DNA-helicase activity and 3'->5' exonuclease activity towards double-stranded DNA with a 5'-overhang.
Molecular interactor	**Molecular Function**
APEX1	DNA-(apurinic or apyrimidinic site) lyase; Multifunctional protein that plays a central role in the cellular response to oxidative stress. The two major activities of APEX1 in DNA repair and redox regulation of transcriptional factors.
APTX	Aprataxin; DNA-binding protein involved in single-strand DNA break repair, double-strand DNA break repair and base excision repair. Resolves abortive DNA ligation intermediates formed either at base excision sites, or when DNA ligases attempt to repair non-ligatable breaks induced by reactive oxygen species.
DCLRE1C	Protein artemis; Required for V(D)J recombination, the process by which exons encoding the antigen-binding domains of immunoglobulins and T-cell receptor proteins are assembled from individual V, (D), and J gene segments.
LIG4	DNA ligase 4; Efficiently joins single-strand breaks in a double- stranded polydeoxynucleotide in an ATP-dependent reaction. Involved in DNA non-homologous end joining (NHEJ) required for double-strand break repair and V(D)J recombination.
POLM	DNA-directed DNA/RNA polymerase mu; Gap-filling polymerase involved in repair of DNA double- strand breaks by non-homologous end joining (NHEJ).
PRKDC	DNA-dependent protein kinase catalytic subunit; Serine/threonine-protein kinase that acts as a molecular sensor for DNA damage. Involved in DNA non-homologous end joining (NHEJ) required for double-strand break (DSB) repair and V(D)J recombination.
TDP1	Tyrosyl-DNA phosphodiesterase 1; DNA repair enzyme that can remove a variety of covalent adducts from DNA through hydrolysis of a 3'-phosphodiester bond, giving rise to DNA with a free 3’ phosphate.
XRCC1	DNA repair protein XRCC1; Involved in DNA single-strand break repair by mediating the assembly of DNA break repair protein complexes.

Molecular interactor	Molecular Function
XRCC4	DNA repair protein XRCC4 involves DNA non-homologous end joining (NHEJ) required for double-strand break repair and V(D)J recombination. Binds to DNA and to DNA ligase IV (LIG4).
XRCC5	X-ray repair cross-complementing protein 5; Single-stranded DNA-dependent ATP-dependent helicase. It has a role in chromosome translocation.
XRCC6	X-ray repair cross-complementing protein 6; Single-stranded DNA-dependent ATP-dependent helicase. It has a role in chromosome translocation.

Figure 2d shows the tridimensional reconstruction of the APTX protein using AlphaFold, comparing the resulting predicted wild protein with the resulting truncated protein once the W279* (p.Trp279Ter) mutation occurs. It shows not only the loss of three beta sheets harbored in the C2H2 Znf domain involved in DNA repair, but the effect onto the whole three-dimensional structure of the protein.

Discussion

AOA1 was reported for the first time in 1988 by Aicardi *et al.* with a series of 14 patients with onset symptoms and neurological signs indistinguishable from Ataxia-Telangiectasia (AT) but without evidence of multisystemic involvement.[16] To the best of our knowledge, this report represents the most extensive series of siblings affected with AOA1 in Latin America, adding knowledge to outline the complex phenotype of hereditary ataxias.

From the clinical point of view, the affected cases presented showed several differences in the presentation of the AOA1 clinical phenotype, *i.e.*, **1)** the age of onset of the symptoms for each patient, *i.e.*, 4, 6, 8 and 11; **2)** the presence of dysarthria not associated to the age of the patient (ranging from no dysarthria in one patient to mild-moderate dysarthria in others); **3)** the muscle tone in all patients was normal, except for the youngest of the siblings which presented a lower limb hypotonia; **4)** the muscle trophism amongst the patients showed differences as well, with two patients presenting atrophy of the gastrocnemius, one presenting thenar and gastrocnemius hypotrophy along with hypertrophy of both deltoids and another presenting normal trophism; **5)** two patients presented bilateral foot drop and two did not; **6)** the sensitivity examination showed essential differences with one patient presenting bilateral glove hypoalgesia and another presenting bilateral glove and stocking anesthesia (the other two patients had normal sensitivity and the amount of time since the onset of symptoms had no correlation with these differences in sensitivity); **7)** three patients presented dyskinesia, one of upper limb and face dyskinesia and two only presented face dyskinesia; and **8)** the neurocognitive and psychiatric compromise affecting the younger of the four brothers with temporo-spatial disorientation, impairment of the semantic and phonologic fluency, episodic memory, constructional apraxia, moderate anomia, and low executive function; further he showed signs of depression. This heterogeneous phenotype suggests the effect of

genetic interactions shaping the natural history of AOA1. In this vein, we hypothesize that genetic effects might be prominent over external forces as the family is subject to a primarily homogenous cultural, environmental influx. This phenotypic heterogeneity has been described by other authors elsewhere.[57,58]

Interestingly, some features of the clinical phenotype were relatively homogeneous, *i.e.*, **1)** none of the patients presented myoclonus, seizures, or chorea; **2)** at inspection, it was noted that the three male patients had pectus excavatum, **3)** the strength of all patients was slightly affected in all muscle groups, **4)** all patients presented dysmetria in the finger to eye test; **5)** no patient was found to have cavus foot, a finding reported in several cases of ataxias, and finally **6)** the cranial nerves examination was regular except for the ocular-motor apraxia found in all four patients, a crucial finding for the description of the disease.

It is essential to distinguish the clinical similarities and differences amongst AOA1, spinocerebellar ataxia type 2 (SCA2), and ataxia-telangiectasia (AT),[59,60] these last two diseases being the main differential diagnosis in patients with ataxia and oculomotor apraxia. Similarly, to AOA1, AT is characterized by progressive cerebellar ataxia with an early onset and oculomotor apraxia.[61–63] However, AT patients frequently present choreoathetosis, oculocutaneous telangiectasias, and an immunodeficiency syndrome that predisposes them to frequent non-opportunistic infections and an increased risk for malignancies like leukemia and lymphoma.[16] Non-classic forms of AT include adult-onset AT, AT Fresno variant (which combines features of Nijmegenn Breakage Syndrome), and classic AT.[61–63] Classic AT presents with early-onset dystonia or hypotonia and has been described in extended Mennonite families.[61–63] These patients present a high frequency of cancer and adverse responses to chemotherapeutic agents, progressive dystonia as a presenting manifestation, adult-onset spinal muscular atrophy associated with reduced levels of ATM protein, and Breast cancer followed by late-onset AT.[63] On the other hand, SCA2 has an autosomal dominant inheritance pattern characterized by progressive cerebellar ataxia with nystagmus and slow saccadic eye movements. Sometimes ophthalmoparesis or levodopa-responsive parkinsonism may be present. It has an age of onset in the fourth decade of life with ten to fifteen years of disease duration but is more rapidly progressive when onset occurs before age 20. Also, deep tendon reflexes are brisk early on and absent later in the course. Peripheral neuropathy and intellectual impairment may be present, and myoclonus, dystonia, and chorea are less common but have been described.[61,63–65]

Interestingly, the application of the STRING protein interaction tool shows different molecules interacting with and related to APTX. Indeed, the APTX protein interacts with the ATM protein, whose genes, when mutated, produce clinical conditions with similar phenotypes (AOA1 and AT, respectively), suggesting that this interaction is essential in producing the phenotypes of both diseases **(**Figure 2B**).**[66] Similarly, the APTX protein also interacts with the XRCC4, XRCC1, and TDP1 proteins whose genes, when mutated, give rise to clinical entities that also present ataxia, suggesting the critical value of these interactions in the standard features of these clinical entities.

In a review of 22 Portuguese patients from 11 kindreds, 14 (64%) patients had progressive external ophthalmoplegia, 13 (59%) dystonia, 9 (41%) cavum foot, and 6 (27%) scoliosis; 86% had an onset of the disease before early childhood, and the rest having an onset at 10, 15 and 16 years old. All patients examined had generalized areflexia. None of them had mental retardation, telangiectasia, and immunodeficiency.[59] Cerebellar atrophy was a consistent finding without correlation with both brainstem involvement and disease length.

In a series of fourteen patients, from nine families with AOA1, with five different mutations in the *APTX* gene, the W279X nonsense mutation (837G->A, exon 6) was the more frequent in this series. Interestingly, the clinical phenotype of W279X homozygous patients is very classical, contrasting with the clinical phenotype of A198V homozygous patients affected with diffuse choreic movements and with an onset of symptoms since the two y/o.[67] An atypical presentation of AOA1 describes a three y/o child with poor balance when walking, without oculomotor apraxia, chorea, or cerebellar atrophy, and a confirmed W279X mutation.[68] Similarly, a Hispanic AOA1 was affected with an Alu-mediated mechanism responsible for a deletion of the exon 6, but curiously with lack of oculomotor apraxia.[69]

Later studies have focused on specific variations in blood, muscle biopsy, or nerve conduction, trying to find beneficial interventions for patients. Hypoalbuminemia and hypercholesterolemia are frequently found in patients with AOA1.[67,70] Levels of alpha-fetoprotein are normal, contrasting with elevated levels in AOA2.[67,70,71] The Coenzyme Q10 deficiency has been described in AOA1 patients, and its supplementation has been proposed as an intervention to delay the muscular atrophy; however, there is contradictory evidence.[72,73]

As mentioned above, the family lived in a very remote zone of Colombia, a rural community in the state of Magdalena, Colombia. This made it impossible to transfer all of them to a city where formal diagnostic studies could be carried out; so only clinical examinations and genetic tests were carried out. The geographical location is of complicated public access, and the family's homes are on rocky soil, making it very difficult for the patients to have adequate physical support when struggling with their disease. The family struggles to access essential health services, drinking water, and an appropriate diet. All family members have not attended school, have a history of exposure to biomass, and are illiterate.

The mutation described herein has not been reported in other studies from Latin America, which suggests a founder effect in this population. Given that AOA1 is inherited in an autosomal recessive manner, at conception, each sib of an affected individual has a 25% chance of being affected, a 50% chance of being an asymptomatic carrier, and a 25% chance of being neither affected nor a carrier. Carrier testing for at-risk family members and prenatal testing for pregnancies at increased risk are possible if both pathogenic variants in a family have been identified.

Declarations

Research involving Human Participants

Ethics approval: All procedures performed in our study involving human participants met the ethical standards of the institutional research committee (Comité de Ética of the University of Antioquia, School of Medicine), Project: “Biobanco de Investigación, Neurobanco”, code F-017-00, approval resolution #006 (reviewed amendment date of approval May 13, 2021), and with the 1964 Helsinki declaration and its later amendments.

Disclosure of potential conflicts of interest: The authors declared they have no conflict of interests to disclose.

Consent to participate: Informed consent was obtained from all individual participants included in the study. Parents provided informed consent for their daughter (younger than 16 years of age). The used informed consent, and those signed by patients and family members are available upon request.

Consent for publication: The informed consent signed by the participants includes a section that allows the publication of research findings.

Availability of data and materials: Data and materials described in this manuscript are available upon request of researchers.

Competing interests: The authors declared they have no financial interests to disclose.

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Authors' contributions: Conceptualization, F.L. and M.A-B.; methodology, F.L., D.A., D.V., L.M., S.M., D.H., M.I-R., J.J.L., I.L.,VN-S., C.M.R., O.M.V., J.I.V., M.A-H., and M.A-B.; software, I.L.,VN-S., O.M.V., J.I.V., M.A-H., and M.A-B.; validation, J.I.V. and M.A-B.; formal analysis, D.A., S.M., D.H., I.L.,VN-S., O.M.V., J.I.V., M.A-H., and M.A-B.; investigation, F.L., D.A., D.V., L.M., S.M., D.H., M.I-R., J.J.L., I.L.,VN-S., C.M.R., O.M.V., J.I.V., M.A-H., and M.A-B.; resources, F.L., M.I-R., J.J.L., I.L.,VN-S., J.I.V., and M.A-B.; data curation, D.A., D.V., L.M., S.M., D.H., M.A-H., and M.A-B.; writing—original draft preparation, D.A., D.V., M.A-H., and M.A-B.; writing—review and editing, F.L., D.A., D.V., L.M., S.M., D.H., M.I-R., J.J.L., I.L.,VN-S., C.M.R., O.M.V., J.I.V., M.A-H., and M.A-B.; visualization, D.A., D.V., L.M., I.L.,VN-S., O.M.V., J.I.V., M.A-H., and M.A-B.; supervision, F.L. and M.A-B.; project administration, F.L. and M.A-B.; funding acquisition, F.L., M.I-R., J.J.L., I.L.,VN-S., J.I.V., and M.A-B.; All authors have read and agreed to the published version of the manuscript.

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Figures

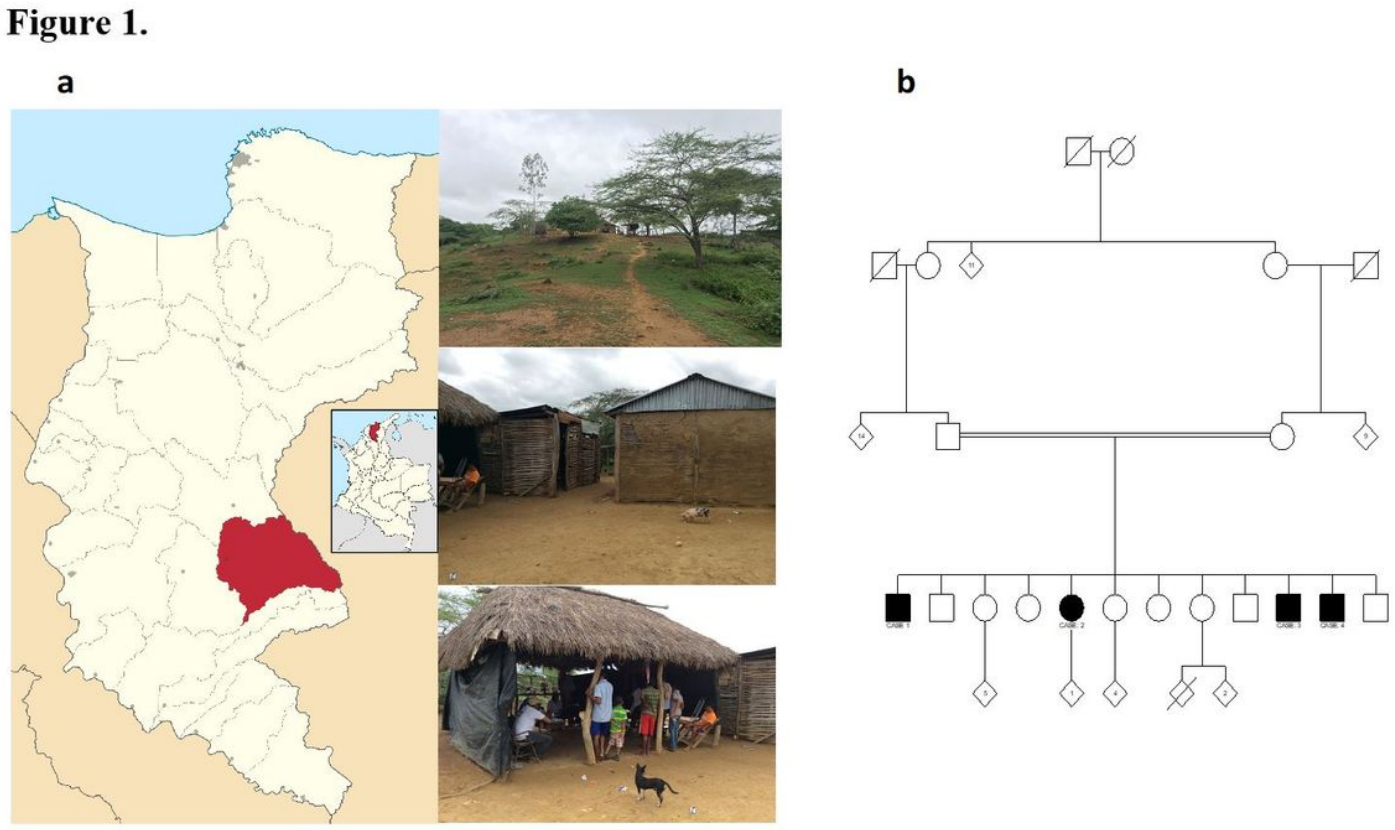

Figure 1

a) Location of the municipality of “Ariguaní”, the town of “El Difícil”, in the State of Magdalena, Colombia (in the small caption, Colombia location in South America, limited to the north with the Caribbean Sea). The family lived in a very remote zone of Colombia, so it was impossible to transfer them to a city where formal diagnostic studies could be carried out, so only clinical examinations and genetic tests were done. There is no road available for motorized vehicles, and most of the travel is made by horse or walking. b) Four generations consanguineous pedigree with Ataxia with Ocular Apraxia type 1 (AOA1) in Four

Siblings (APTX, W279*, p.Trp279Ter mutation). Both parents are unaffected, and the four affected individuals are immersing in a sibship of 12 sibs. There are twelve descendants in the last generation. The geographical barriers limit emigration and immigration because the territory is plagued by guerrillas, paramilitary groups, and narcotics smugglers. The geographical location is challenging for public access, and the family's homes are on rocky soil, making it very difficult for the patients to have adequate support when struggling with their disease. The family struggles to be able to access basic health services, drinking water and an appropriate diet. All members of the family have not attended school, have history of exposure to biomass, and are illiterate.

Figure 2.

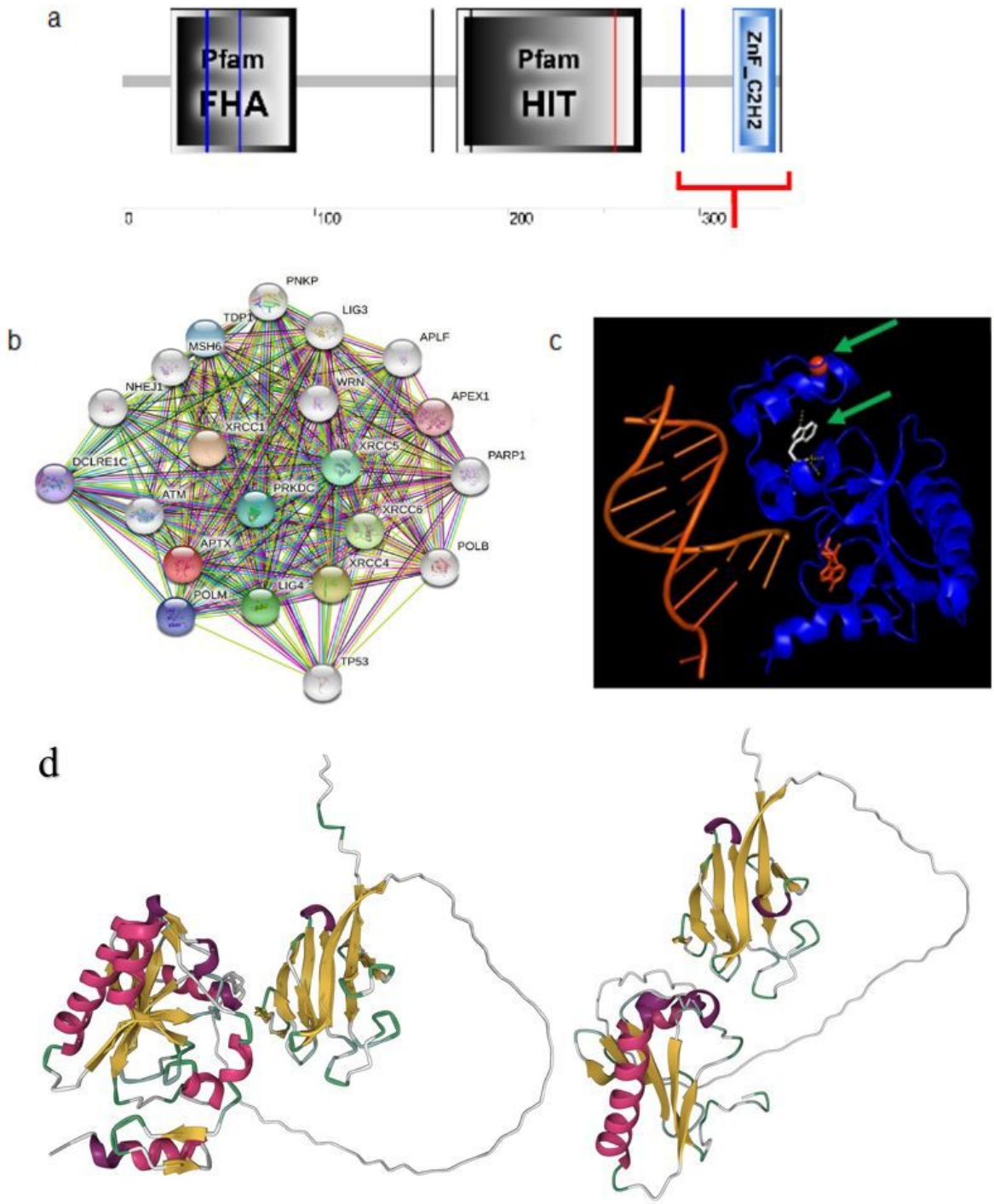

Figure 2

a) Schematic view of APTX protein, FHA, HIT and C2H2-type (classical) zinc fingers (Znf), see red bracket. b) STRING protein interaction tool showing different molecules interacting with and/or related to APTX. c) PyMOL modeling of APTX protein showing the Trp279 specific region (green arrows) and the Zinc withing the zinc finger domain (in red). d) tridimensional reconstruction of the APTX protein using AlphaFold, comparing the resulting predicted wild protein (left) with the resulting truncated protein (right)

once the W279* (p.Trp279Ter) mutation occurs, showing not only the loss of three beta sheets harbored in the C2H2 Znf domain involved in DNA repair, but the effect onto the whole three dimensional structure of the protein.