Movement Disorders
CLINICAL PRACTICE

CASE SERIES

Mild Neurological Phenotype Associated with Hypomorphic Variants in the Ataxia-Telangiectasia Mutated Gene

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ABSTRACT: Background: Ataxia-telangiectasia (A-T) is a progressive multisystemic neurodegenerative disease. The phenotypic spectrum includes conditions (variant A-T) with mild, late-onset, and atypical clinical presentations characterized by the prevalence of dyskinetic rather than ataxic features.
Cases: We describe the clinical presentations of 3 siblings with early-onset truncal ataxia without obvious neurological deterioration or biological markers of classic A-T phenotype. We performed functional and genetic evaluation of 3 siblings with very mild neurological phenotype. Genetic evaluation with a next-generation sequencing panel for genes causative of cerebellar ataxia detected 2 known ATM gene variants, missense c.9023G>A p.(Arg3008His), and leaky splicing c.1066-6T>G variants. Functional studies showed mildly reduced ATM expression and residual kinase activity in the probands compared with healthy controls.
Conclusions: These results suggest the importance of investigating ATM variants even in the presence of clinical and biological atypical cases to ensure specific therapeutic regimens and oncological surveillance in these patients.

Ataxia-telangiectasia (A-T) is a rare autosomal recessive neurodegenerative disorder. Classic A-T forms are characterized by early-onset progressive cerebellar ataxia resulting in the loss of autonomous walking by the age of 10, oculomotor apraxia, oculocutaneous telangiectasia, cancer susceptibility, and immunodeficiency. Exitus occurs by the second or third decade of life due to chronic lung disease or malignancies. Increased alpha-fetoprotein (AFP), immunodeficiency, and radiosensitivity are typical biomarkers. In the past 20 years, expanded A-T phenotypes (variant A-T) have been identified, which are characterized by late-onset, mild, or absent cerebellar involvement, prevalent dyskinetic movement disorders, and mild/absent systemic features.[1]

A-T is caused by biallelic variants in the A-T mutated (ATM) gene, encoding for a Phosphatidyl Inositol 3-kinase-related serine/threonine protein kinase involved in DNA damage response (DDR). In classic A-T, ATM nonsense or frameshift truncating variants lead to undetectable ATM expression and consequent loss of kinase activity. In variant A-T, splice site or missense ATM variants are commonly associated with residual ATM expression and kinase activity.[2]

Here, we report 3 brothers with early-onset nonprogressive truncal ataxia without any other key diagnostic features of A-T. Their ATM genotype included compound heterozygosity for missense c.9023G>A p.(Arg3008His) and splicing c.1066-6T>G variants.

Case Series

Case 1 (Proband 1)

This 16-year-old boy was born after a normal pregnancy and delivery from healthy unrelated parents. At the age of 24 months,

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Keywords: variant ataxia-telangiectasia, mild phenotype, functional studies, p53-MCL, kinase activity.
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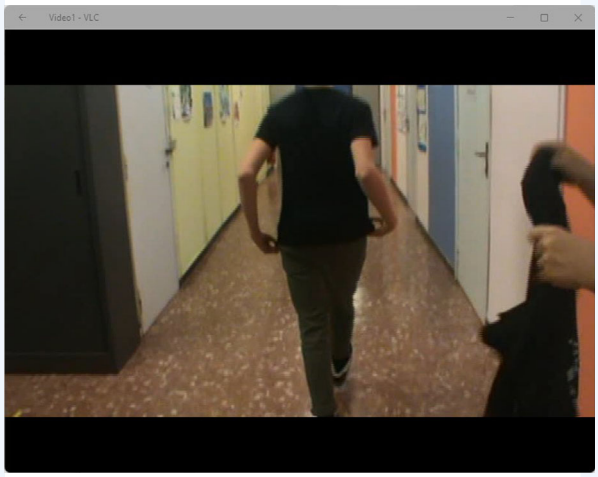
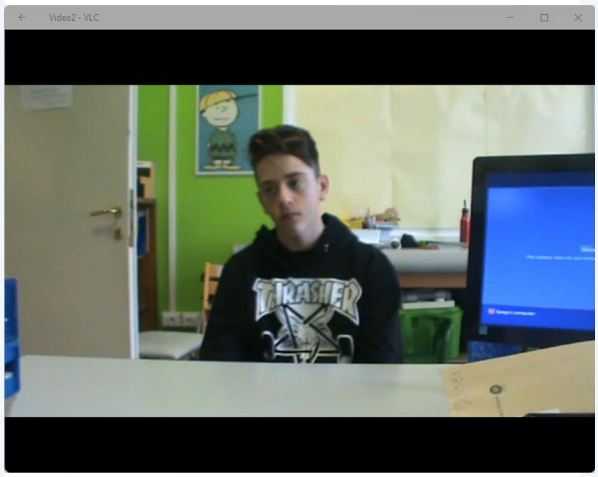


Video 1. Normal gait with normal speed; dystonic posturing of the upper limbs.
Video content can be viewed at https://onlinelibrary.wiley.com/doi/10.1002/mdc3.13618



Video 2. Sitting position with slight oscillation of the trunk.
Video content can be viewed at https://onlinelibrary.wiley.com/doi/10.1002/mdc3.13618

trunk swaying was noticed. Slight cerebellar ataxia was diagnosed at the age of 9 years and brain magnetic resonance imaging (MRI) examination disclosed cerebellar shrinking. On examination at the age of 14, he was an intelligent boy with a very slight trunk ataxia that was prevalent during static rather than dynamic posture, unsteady tandem gait, inconstant dystonic posturing of the upper limbs, very mild intentional tremor and dysmetria, and hyporeflexia of the lower limbs (Videos 1 and 2); neurophysiological assessment excluded axonal peripheral neuropathy. Eye movements were preserved with externally beating nystagmus in extreme eccentric eye positions. No variation was detected on brain MRI.

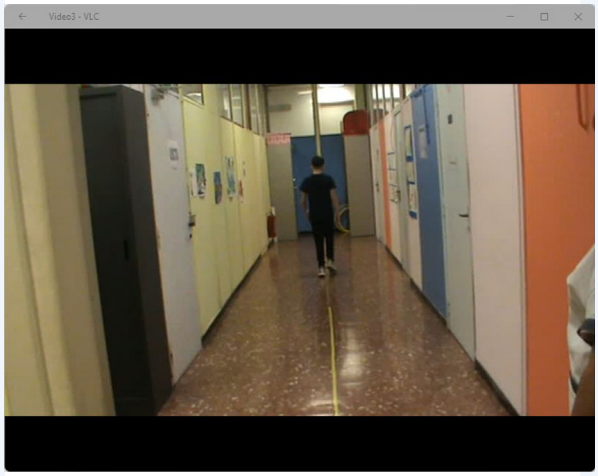
Case 2 (Proband 2)

The 14-year-old brother of proband 1 (P1) was born after a normal pregnancy and delivery. At the age of 24 months, trunk swaying was noticed. At the age of 7, he was described as slightly ataxic. Brain MRI disclosed cerebellar shrinking. Cognitive development was normal. On examination at the age of 11, he exhibited, similar to his older brother, slight truncal ataxia, dystonic posturing of the upper limbs, slurred speech, and lower limb hyporeflexia (Videos 3 and 4). Axonal peripheral neuropathy was ruled out. Ocular motility was unaffected. At the age of 12, brain neuroimaging was unchanged.

Case 3 (Proband 3)

The youngest brother, aged 8 years, was born after a normal pregnancy and delivery. Psychomotor and neurological development had always been considered normal by the parents. He was examined at the age of 8 during a control of his two older brothers. He showed very mild choreic movements of the upper limbs, clumsiness, and unsteady tandem gait.

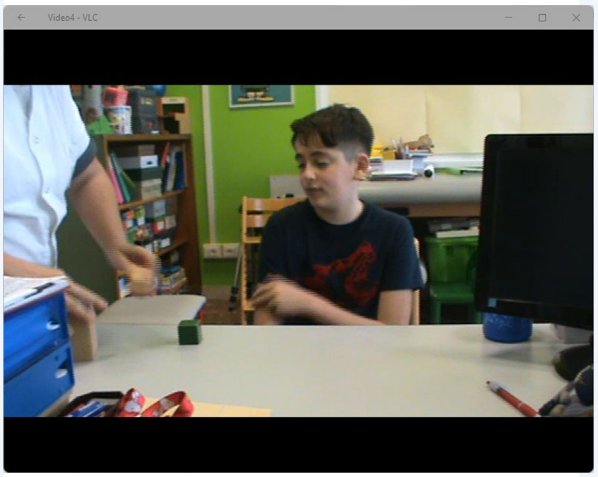
Diagnostic workup of the 3 brothers showed normal AFP and immunoglobulin levels; no clinical progression was evident.



Video 3. Normal gait with normal speed; dystonic posturing of the upper limbs.
Video content can be viewed at https://onlinelibrary.wiley.com/doi/10.1002/mdc3.13618

Genetic Results

The next-generation sequencing panel for ataxias and subsequent filtering detected 2 known variants in the ATM gene: a maternally derived splicing site variant, c.1066-6T>G (rs201686625),



Video 4. Sitting position with slight oscillation of the trunk. Video content can be viewed at https://onlinelibrary.wiley.com/doi/10.1002/mdc3.13618

and a paternally derived missense variant, c.9023G>A p.(Arg3008His) (rs587781894). Segregation analysis demonstrated that the 3 affected siblings were compound heterozygotes (Table 1).

Functional Studies

ATM protein expression and activity were evaluated on freshly isolated and reactivated peripheral blood mononuclear cells (PBMCs) from the probands (proband 1 [P1], proband 2 [P2], and proband 3 [P3]), their parents (mother [M] and father [F]), and 1 healthy control (HD). Compared with the HD, reduced but clearly detectable levels of full-length ATM proteins were observed in the probands and their parents (Fig. 1A,B). Partial kinase activity was also detected upon γ-irradiation of PBMCs, as shown by $ATM^{Ser1981}$ autophosphorylation and activation of downstream DDR targets. The protein 53- p53-MCL test (Fig. 1C), which identifies the presence of defective ATM protein through impaired p53 localization at centrosomes in mitosis,[3] showed only a partial reduction in the 3 siblings (P1 = 64%, P2 = 63%, P3 = 58%) compared with the controls (HD = 82%, M = 68.5%, F = 74%).

Discussion

We report 3 brothers affected by variant A-T presenting with early-onset truncal ataxia without neurological deterioration. Other characteristic features of the disease, such as oculomotor apraxia, telangiectasia, and immunodeficiency, were absent. Truncal ataxia is the most frequent presenting symptom in classical A-T, usually at approximately 9 months of age as observed in the presymptomatic siblings of the affected patients.[4] The

TABLE 1 Classifications and predictions of all ATM variants found in the family

Variant	Molecular Consequence	dbSNP Identifier	Classification					Prediction			CADD Score	Allele Frequency	SpliceSite Prediction VarSEAK (Δscore)
			P1	P2	P3	M	F	ClinVar	VarSome	Provean	Sift	Mutation Taster		gnomAD	
c.1066-6T>G	Intronic	rs201686625	htz	htz	htz	htz	/	Conflicting: B/LB/VUS[a]	VUS	N/A	N/A	Polymorph.	20.9	G = 0.001385	−38.36%
c.9023G>A	p.(Arg3008His)	rs587781894	htz	htz	htz	/	htz	LP[a]	P	Deleterious	Damaging	Disease causing	30	A = 0.000007	+0.00%
c.5948G>A[b]	p.(Ser1983Asn)	rs659243	hmz	hmz	hmz	hmz	hmz	B	LB	Neutral	Tolerated	Polymorph.	5.097	N/A	+0.00%
c.3342G>A[b]	p.(Lys1114=)	rs138393322	htz	htz	htz	/	htz	Conflicting: B/LB/VUS	LB	Neutral	Tolerated	Disease causing	7.504	A = 0.000442	+0.00%
c.7248C>T[b]	p.(Leu2416=)	rs750513866	htz	htz	htz	/	htz	LB	LB	Neutral	Tolerated	Disease causing	4.459	T = 0.000004	+0.00%

The variants are reported according to Human Genome Variation Society nomenclature guidelines (https://varnomen.hgvs.org/). RefSeq (reference sequence) used for variant annotation: NM_000051.4.
[a]During the revision of the manuscript, ClinVar updated variant c.1066-6T>G classification from conflicting to benign, and the variant c.9023G>A classification from likely pathogenic to pathogenic/likely pathogenic.
[b]Filtering permitted the exclusion of synonymous variants not affecting splicing and variants with frequency >1% in human variation databases.
Abbreviations: ATM, Ataxia-Telangiectasia Mutated; P1, proband 1; P2, proband 2; P3, proband 3; M, mother; F, father; CADD, Combined Annotation Dependent Depletion; htz, heterozygous; N/A, not available; hmz, homozygous; B, benign; LB, likely benign; VUS, variant of uncertain significance; LP, likely pathogenic; P, pathogenic; Polymorph., polymorphism

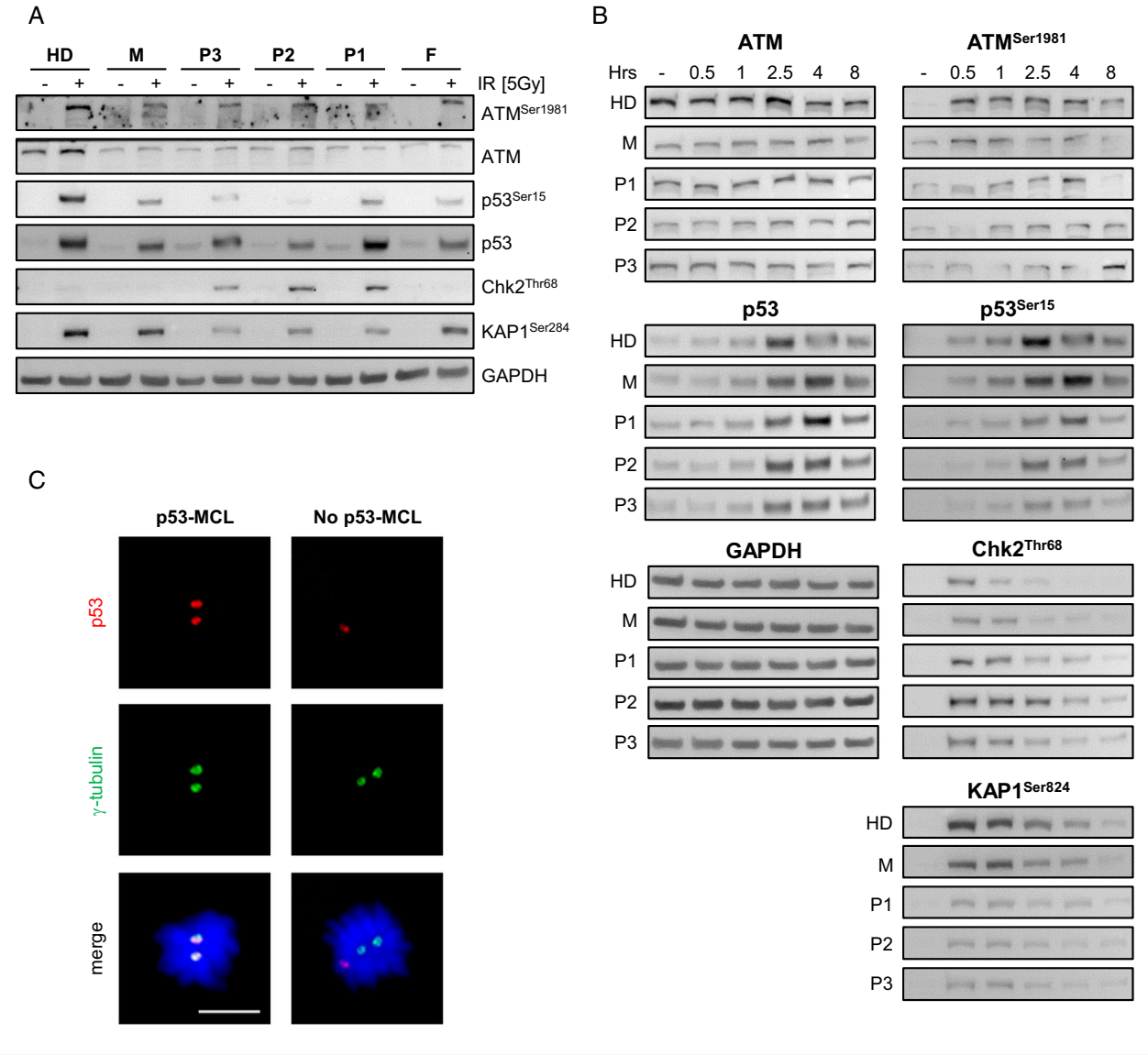


FIG. 1. Residual ATM expression and kinase activity are maintained in patients affected by variant ataxia-telangiectasia. **(A, B)** WBs of peripheral blood mononuclear cell (PBMC) lysates from the indicated donors before (−) and after 2.5 hours (+) irradiation (A) or at the indicated timepoints after 2 Gy irradiation (B). GAPDH was used as loading control. **(C)** Representative images (100×) of mitotic PBMCs with or without localization of p53 (red) at centrosomes (green). Scale bar = 5 μm. F, father; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; HD, healthy control; M, mother; P1, proband 1; P2, proband 2; P3, proband 3.

emergence in the following months of typical signs of cerebellar impairment confirms the nature of this sign as ataxic. However, similar trunk oscillations may reflect the motor impersistence that characterizes chorea and therefore a possible anticipatory symptom of a dystonic-dyskinetic presentation of the disease. Lacking any other sign of progression either toward cerebellar ataxia or toward a dyskinetic form, the term *truncal ataxia* in these subjects has a description rather than an anatomical localization value.

The clinical spectrum of A-T includes many different phenotypes, including mild forms, late presentations, and predominantly dyskinetic presentations (Table S1). In some cases, typical A-T markers, such as AFP and immunoglobulin defects, are absent. In other cases, there is a similarly mild phenotype even though all A-T cellular biomarkers are present (ie, radiosensitivity, chromosomal rearrangements, undetectable ATM protein, and kinase activity) (Table S1). In a girl with an apparent classic presentation associated with typical A-T biological markers, a remitting form was described.[5] These aspects highlight the occurrence of other modifying factors that may influence clinical outcome.

It has been proposed that mild clinical impairment is related to residual protein expression and kinase activity associated with missense and splice-site ATM variants.[2] Accordingly, the

genotype of the 3 siblings disclosed the missense ATM variant c.9023G>A p.(Arg3008His) and the leaky splicing variant c.1066-6T>G, which leaves residual full-length ATM RNA.[6] Moreover, the biochemical and functional assays we performed on their PBMCs showed that the ATM protein maintains residual expression and kinase activity on both DDR and p53-MCL. This genotype was previously described in a 45-year-old man with variant A-T.[2] Based on their classificatory algorithm, the authors assigned this patient to the A-T neurological phenotype group A (ie, cerebellar ataxia and/or peripheral neuropathy with minimal or no extrapyramidal involvement) and genetic group 1 (ie, some normal ATM protein, with residual kinase activity present, attributed to a leaky splice site mutation).[2] High ATM protein levels in the patient's lymphoblastoid cells and low AFP levels were consistent with the less severe presentation in this patient.[2] These aspects correlate with the genotype–phenotype observed in our patients, supporting the idea that milder neurological symptoms reflect retained ATM expression and kinase activity.

The association of c.9023G>A p.(Arg3008His) with a different variant (c.2304_2305insTT p.Glu769Leufs*9) was reported in a 35-year-old woman with cervical and ovarian carcinomas and sensorimotor axonal polyneuropathy.[7] She complained of walking and learning difficulties from the age of 9 and became wheelchair bound at the age of 23. Blood examination detected low immunoglobulin A and immunoglobulin G and high AFP levels. Brain MRI revealed cerebellar atrophy. A-T diagnosis followed that of cancer, highlighting the potential risk of missed or delayed diagnosis in patients with variant A-T. Moreover, these findings suggest that oncogenesis and neurological impairment in A-T are probably the result of different pathogenic mechanisms.

Although both c.9023G>A p.(Arg3008His) and c.1066-6T>G variants have been described in A-T and cancer patients,[2,6–10] the pathogenicity of c.1066-6T>G is still debated (Table 1). This variant is relatively common in the general population[11,12] and has leaky splicing activity,[6,10] and bioinformatics tools predict it is not likely to severely affect the splice acceptor site.[13] Doubts regarding c.1066-6T>G pathogenicity have also been raised by the observation that A-T patients originally reported as biallelic c.1066-6T>G homozygotes carried an additional ATM truncating mutation[13] or the splicing c.967A>G p.(Ile323Val) variant.[12] However, our study and other clinical reports support the pathogenicity of this ATM variant. In particular, Schröder and colleagues described a 27-year-old woman with ocular motor apraxia since the age of 4 months, early-onset ataxia without deterioration, no telangiectasia or immunological alterations, and normal brain MRI at the age of 19. She was compound heterozygous for the c.1066-6T>G variant and the silent c.2250G>A p.(Lys750=) variant affecting splicing.[6] ATM protein expression and kinase activity in lymphoblastoid cells were reduced to approximately 10% to 30%. Compound heterozygosity for the c.1066-6T>G and missense c.9022C>T p.(Arg3008Cys) variants (the same codon of our variant but a different residue, see next paragraph) was detected in 3 subjects. A 48-year-old patient with multiple myeloma showed a mild A-T phenotype and retained ATM kinase activity ascribed to the c.1066-6T>G variant.[14] The same genotype was detected in 2 sisters. The older 24-year-old sister presented with progressive ataxia and choreoathetosis of the arms and neck. Her 20-year-old sister suffered from focal dystonia with retained autonomous walking. She showed ocular telangiectasia, whereas no immunodeficiency was detected and the AFP value was normal.[15] Despite the lack of definite data, they overall suggest a pathogenic tendency of the c.1066-6T>G variant. However, to avoid the potential contribution of concomitant polymorphisms and/or classically considered benign variants, such as those found in our family (Table 1), more genetically controlled assays, such as the generation of variant-specific cells by genome editing, are mandatory.

Less concerns are linked to the c.9023G>A p.(Arg3008His) variant, classified as likely pathogenic based on (1) its presence in patients with A-T or cancer[2,8]; (2) its presence in a hotspot where 2 additional missense variants, c.9022C>T p.(Arg3008Cys) and c.9023G>T p.(Arg3008Leu), have been found[14,16]; and (3) the generation of a mouse model with impaired kinase activity and tumor susceptibility.[17]

In conclusion, our data confirm that A-T can be underdiagnosed in patients with a mild phenotype and without typical biological markers. Thus, A-T diagnosis should be considered in all patients with movement disorders. Early diagnosis is critical for malignancy surveillance and treatment because radiotherapy and some chemotherapy approaches can cause secondary complications. From a biochemical point of view, functional analyses are needed to better understand variant consequences on ATM in borderline cases, thus clarifying variant contribution to A-T and tumor development.

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Author Roles

1. Research Project: A. Conception, B. Organization, C. Execution; 2. Data Collection and Statistical Analysis: A. Design, B. Execution, C. Review and Critique; 3. Manuscript Preparation: A. Writing of the First Draft, B. Review and Critique.

C.C.: 1A, 1B, 1C, 2A, 2B, 2C, 3A, 3B
G.F.: 1A, 1B, 1C, 2A, 2B, 2C, 3A, 3B
L.T.: 1C, 3B
M.P.: 1A, 2A, 2B, 2C, 3B
E.B.: 2B, 3B
S.S.: 1A, 1B, 1C, 2A, 2B, 2C, 3B
G.Z.: 1A, 1B, 1C, 2A, 2B, 2C, 3B
V.L.: 1A, 1B, 1C, 2A, 2B, 2C, 3B

Disclosures

Ethical Compliance Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and

approved by the Ethics Committee of Bambino Gesù Children's Hospital 19/03/2019 (1779_OPBG_2019) and by Istituti Fisioterapici Ospitalieri (IFO) Ethics Committee (study approval CE/160/09 and subsequent amendments and additions by the Central Ethics Committee of Istituto di Ricovero e Cura a Carattere Scientifico- IRCCS- Lazio). Written informed consent for publication was obtained from the subjects involved in the study. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this work is consistent with those guidelines.

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Financial Disclosures for the Previous 12 Months: The authors declare that there are no additional disclosures to report. ■

References

1. Rothblum-Oviatt C, Wright J, Lefton-Greif MA, McGrath-Morrow SA, Crawford TO, Lederman HM. Ataxia telangiectasia: a review. *Orphanet J Rare Dis* 2016;11(1):159.
2. Schon K, van Os NJH, Oscroft N, et al. Genotype, extrapyramidal features, and severity of variant ataxia-telangiectasia. *Ann Neurol* 2019;85(2):170–180.
3. Prodosmo A, De Amicis A, Nisticò C, et al. p53 centrosomal localization diagnoses ataxia-telangiectasia homozygotes and heterozygotes. *J Clin Invest* 2013;123(3):1335–1342.
4. Leuzzi V, Elli R, Antonelli A, Chessa L, Cardona F, Marcucci L, Petrinelli P. Neurological and cytogenetic study in early-onset ataxia-telangiectasia patients. *Eur J Pediatr* 1993;152(7):609–612.
5. Leuzzi V, D'Agnano D, Menotta M, Caputi C, Chessa L, Magnani M. Ataxia-telangiectasia: A new remitting form with a peculiar transcriptome signature. *Neurol Genet* 2018;4(2):e228.
6. Schröder S, Wieland B, Ohlenbusch A, et al. Evidence of pathogenicity for the leaky splice variant c.1066-6T>G in ATM. *Am J Med Genet A* 2020;182(12):2971–2975.
7. Cao J, Tan RYC, Li ST, et al. Identifying ataxia-telangiectasia in cancer patients: Novel insights from an interesting case and review of literature. *Clin Case Rep* 2021;9(2):995–1009.
8. Paglia LL, Laugé A, Weber J, et al. ATM germline mutations in women with familial breast cancer and a relative with haematological malignancy. *Breast Cancer Res Treat* 2010;119(2):443–452.
9. Thompson D, Antoniou AC, Jenkins M, et al. Two ATM variants and breast cancer risk. *Hum Mutat* 2005;25(6):594–595.
10. Dörk T, Bendix R, Bremer M, et al. Spectrum of ATM gene mutations in a hospital-based series of unselected breast cancer patients. *Cancer Res* 2001;61(20):7608–7615.
11. Broeks A, Urbanus JH, de Knijff P, et al. IVS10-6T>G, an ancient ATM germline mutation linked with breast cancer. *Hum Mutat* 2003;21(5):521–528.
12. Fiévet A, Bellanger D, Rieunier G, et al. Functional classification of ATM variants in ataxia-telangiectasia patients. *Hum Mutat* 2019;40(10):1713–1730.
13. Tavtigian SV, Oefner PJ, Babikyan D, et al. Rare, evolutionarily unlikely missense substitutions in ATM confer increased risk of breast cancer. *Am J Hum Genet* 2009;85(4):427–446.
14. Austen B, Barone G, Reiman A, et al. Pathogenic ATM mutations occur rarely in a subset of multiple myeloma patients. *Br J Haematol* 2008;142(6):925–933.
15. Albertyn C, Cawley N, Taylor ATM, Srinivasan V, Last J I, Murphy RP. P0G02 Variant ataxia telangiectasia in sibling with normal α- fetoprotein levels. *J Neurol Neurosurg Psychiatry* 2010;81:e48. https://doi.org/10.1136/jnnp.2010.226340.124.
16. Feliubadaló L, Moles-Fernández A, Santamariña-Pena M, et al. A collaborative effort to define classification criteria for ATM variants in hereditary cancer patients. *Clin Chem* 2021;67(3):518–533.
17. Milanovic M, Houghton LM, Menolfi D, et al. The cancer-associated ATM R3008H mutation reveals the link between ATM activation and its exchange. *Cancer Res* 2021;81(2):426–437.

Supporting Information

Supporting information may be found in the online version of this article.

Appendix S1. Material and methods.

Table S1. List of atypical A-T cases reported in the literature.