Case report
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The importance of functional analysis: a cautionary case of cerebellar ataxia

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Introduction. Cerebellar ataxias are a heterogeneous group of disorders with various cerebellar and extracerebellar manifestations. The underlying aetiology in early-onset, progressive, sporadic ataxia, is often autosomal recessive cerebellar ataxia (ARCA). The advent and rapid clinical integration of next-generation sequencing (NGS) has made it increasingly possible to provide a genetic diagnosis for patients with suspected ARCA. However, one of the greatest challenges of NGS is the interpretation and reclassification of variants of uncertain significance (VUS).

Case report. Ataxia telangiectasia was suspected due to progressive teenage-onset ataxia in a 42-year-old woman with a history of breast cancer, ovarian mass, and elevated alpha-fetoprotein and CA-125. ATM sequencing demonstrated a homozygous missense VUS. However, functional studies clarified that this VUS was not pathogenic, but there was reduction in senataxin. This enabled clarification that the diagnosis was ataxia with oculomotor apraxia type 2.

Conclusion. Our case highlights the importance of functional studies, where possible, to enable reclassification of VUSs.

Keywords: ataxia, DNA mutational analysis, ataxia telangiectasia mutated (ATM) protein, senataxin (SETX), biomarkers

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L'importance de l'analyse fonctionnelle : un cas significatif d'ataxie cérébelleuse

Introduction. Les ataxies cérébelleuses constituent un groupe hétérogène de troubles, présentant diverses manifestations cérébelleuses et extracérébelleuses. L'étiologie sous-jacente de l'ataxie sporadique progressive à début précoce est souvent l'ataxie cérébelleuse autosomique récessive. L'apparition et l'intégration clinique du séquençage de nouvelle génération ont rendu possible le diagnostic génétique pour les patients chez qui l'ARCA est suspectée. Cependant, un enjeu important du NGS est l'interprétation et la reclassification des variants de signification incertaine.

Étude de cas. L'ataxie télangiectasique a été soupçonnée en raison d'une ataxie progressive apparaissant à l'adolescence chez une femme de 42 ans ayant des antécédents de cancer du sein, de masse ovarienne, et de taux élevés d'alpha-fœtoprotéine et de CA-125. Le séquençage de l'ATM a mis en évidence un VUS homozygote faux-sens. Cependant, des études fonctionnelles ont permis de préciser que ce VUS n'était pas pathogène, mais qu'il y avait une réduction de la sénataxine. Cela a permis de clarifier le diagnostic d'ataxie avec apraxie oculomotrice de type 2.

Conclusion. Notre cas souligne l'importance des études fonctionnelles, lorsque cela est possible, pour permettre la reclassification des VUS.

Mots-clés : ataxie, analyse mutationnelle de l'ADN, protéine ATM, sénataxine (SETX), marqueurs biologiques

Abbreviations

ARCA: autosomal recessive cerebellar ataxias
NGS: next-generation sequencing
SARA: scale for the assessment and rating of ataxia
SCAR: spinocerebellar ataxias
VUS: variants of uncertain significance

1. Introduction

Autosomal recessive cerebellar ataxias (ARCA)/ Spinocerebellar ataxias (SCAR) are a heterogeneous group of rare conditions, most frequent of which are Friedreich's ataxia and SPG7-associated ataxia [1]. However, of patients with early-onset of symptoms, ataxia telangiectasia (ATX-ATM) is the second most common type [1]. This is a neurodegenerative and immunodeficiency multisystem disorder, characterised by cerebellar ataxia, ocular telangiectasia, oculomotor apraxia, predisposition to recurrent sinopulmonary infections, radiosensitivity. Both patients and heterozygous carriers have increased cancer risk [2]. Pathogenic variants in ATM affecting the level of ATM and activity/signalling capacity of the ATM kinase are responsible for the phenotype [2]. Multiple causative variants have been described; functional studies assessing the presence of retained enzyme activity predict age of onset, clinical symptoms and progression better than the variant nomenclature alone [3]. Patients with retained ATM kinase activity due to leaky splice site or missense variants have a milder phenotype, variant ATX-ATM [3].
ATX-SETX is another early-onset ARCA, caused by pathogenic SETX variants, which shares many similar features with ATX-ATM including oculomotor dysfunction, cerebellar atrophy, sensorimotor axonal neuropathy, chorea and/or dystonia. However, it is not associated with immunodeficiency or predisposition to cancer and telangiectasia is rare. Variants in both SETX and ATM affect DNA repair and contribute to cell death [4].
Here, we report a patient initially suspected to have ATX-ATM due to progressive ataxia with a history of malignancy in association with elevated levels of CA-125 and AFP and homozygous VUS in ATM. However, functional analysis reclassified the ATM VUS to likely benign and identified a reduced level of senataxin, leading to a diagnosis of ATX-SETX.

2. Case description

A 42-year-old Slovakian lady presented with progressive unsteadiness since age 17 years. She had a history of strabismus surgery at 4 years, left temporal haemorrhage at 21 years secondary to an angioma, splenomegaly, prior breast cancer and ovarian mass under investigation by gynaecology at time of referral. There was no family history of ataxia, breast cancer, or consanguinity. On examination she had tortuous conjunctival vessels, gaze-evoked horizontal nystagmus and jerky pursuit movements (Suppl. Video 1). She had cerebellar dysarthria, myoclonic jerks and 4 limb incoordination. Vibration sensation was impaired to the anterior superior iliac spine and reflexes were absent in the lower limbs. She was wheelchair dependent. Scale for the assessment and rating of ataxia (SARA) score was 19.5/40 [5].
MRI brain demonstrated diffuse cerebellar atrophy along with vermian atrophy resulting in enlargement of the 4th ventricule (Figure 1). Neurophysiology confirmed a moderately severe length-dependent sensorimotor axonal neuropathy. Investigations showed elevated AFP at 65.7 IU/mL (normal value <5 IU/mL) and CA-125 at 76 U/mL (normal value <35 U/mL). It was decided to proceed with sequencing of the ATM gene, given the clinical suspicion for ATX-ATM. This demonstrated a homozygous missense variant c.1010G>A p.(Arg337His) in ATM affecting a highly conserved nucleotide and amino acid. This variant is classified by American College of Medical Genetics (ACMG) criteria as a variant of uncertain significance (VUS), although listed on ClinVar as benign, likely benign and VUS [6]. This variant had a minor allele frequency of 0.000079. Both parents were heterozygous. This ATM variant has been suggested as a risk for cancer development [7], raising the initial possibility that both her breast cancer and ataxic syndrome occurred as a consequence of this ATM variant. However, subsequent analysis did not demonstrate cytogenetic chromosomal instability. Furthermore, western blotting revealed a normal level of ATM protein and functional studies assessing differential phosphorylation of proteins demonstrated normal ATM kinase activity/signalling, suggesting that this ATM variant was not pathogenic.

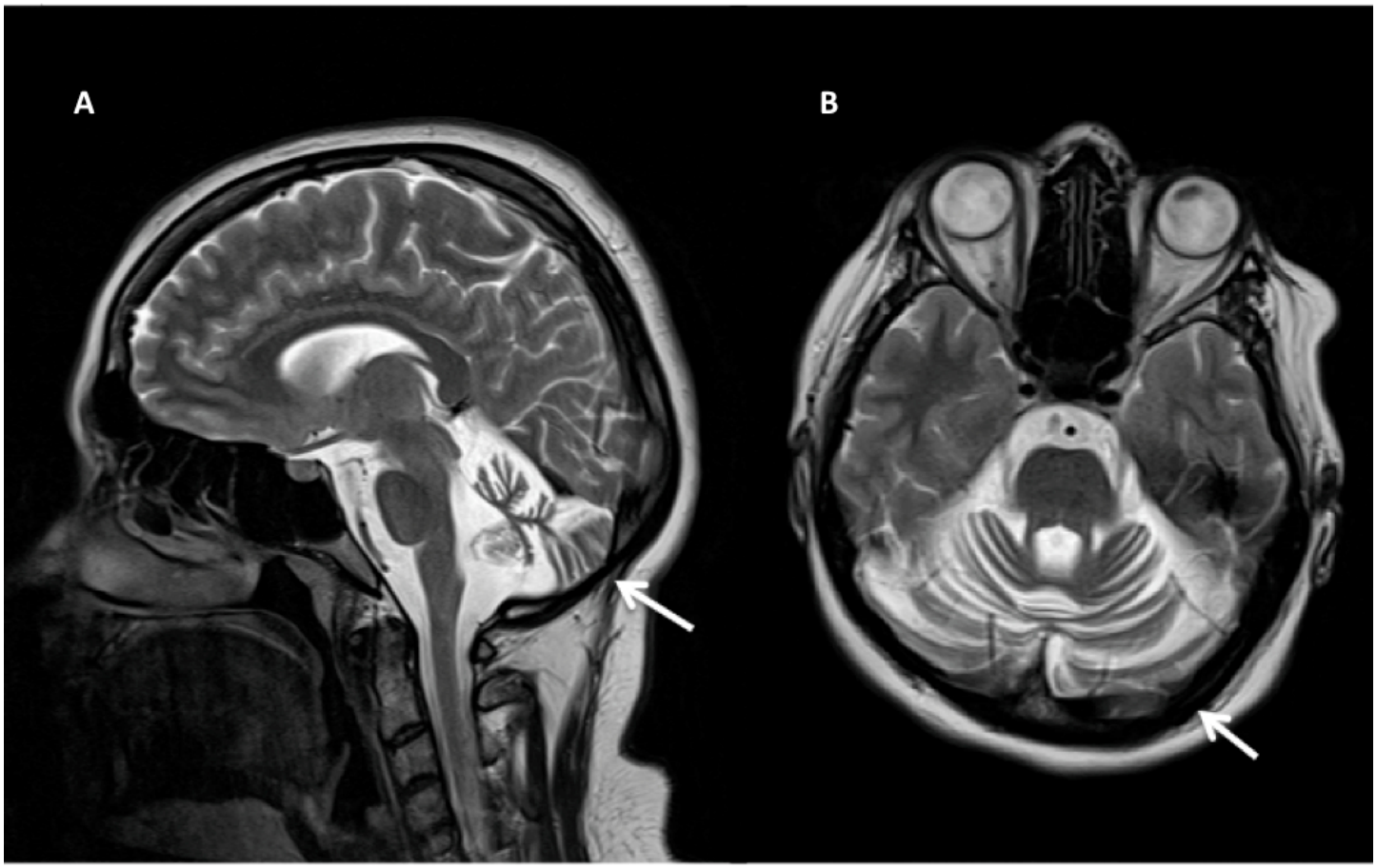

Figure 1. Magnetic resonance brain imaging. Sagittal (A) and axial (B) T2-weighted brain imaging demonstrating diffuse cerebellar atrophy.

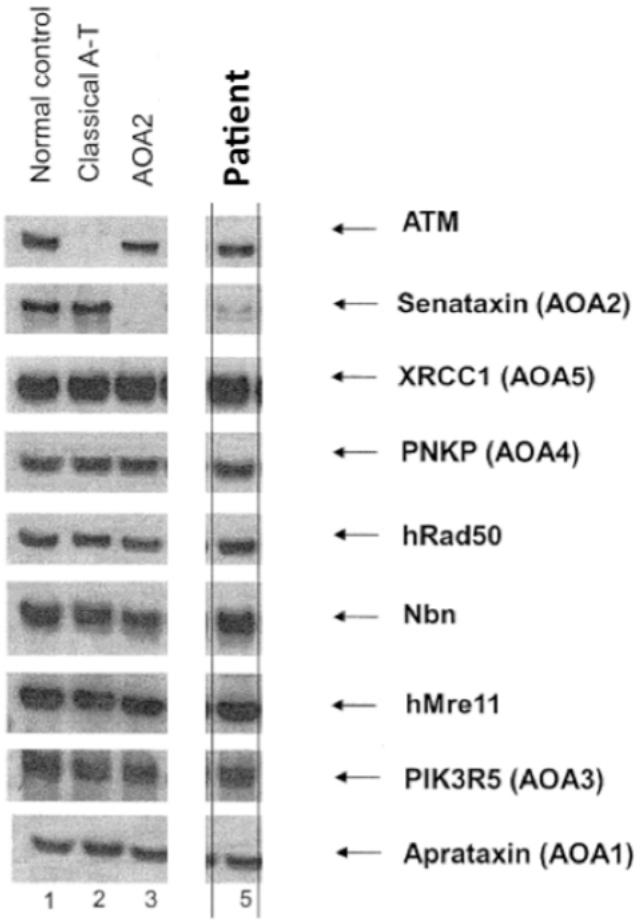

Figure 2. Western blot lysate of the patient's blood (lane 5) demonstrating a reduced level of senataxin protein and preserved level of ATM protein.

Considering the similarities between ATX-ATM and ATX-SETX, Western blotting was performed to assess levels of senataxin, which was confirmed to be reduced (Figure 2). Sequencing of SETX revealed two variants: a previously reported c.5825T>C, p.(Ile1942Thr), and another variant listed in ClinVar c.1484T>C, p.(Leu495Pro) [8-10]. The first variant is located inside the helicase domain and allows some expression of mutant senataxin. It has a minor allele frequency of 0.00001195. Pathogenicity prediction tools, Mutation Taster and Polyphen-2 suggest that the second variant is damaging. The variant was not present in gnomAD. Her father was a heterozygous carrier of p.(Ile1942Thr), and her mother a heterozygous carrier of p.(Leu495Pro), confirming compound heterozygosity in the proband. Both ACMG and ACGS (Association for Clinical Genomic Science) criteria classify both of these variants as pathogenic (Table 1).

Subsequently, her ovarian mass was revealed to be an ovarian endometriotic cyst and benign paratubal serous cystadenoma following investigation through a total abdominal hysterectomy and bilateral salpingo-oophorectomy.

Table 1. Evidence for classification of the variants using ACGM / ACGS guidelines [13,14].

ATM variant c.1010G>A p.(Arg337His) [6]	
Criteria code weight	**Pathogenic evidence**
BS3 Strong	Well-established functional studies show no deleterious effect on protein function
BP1 Supporting	Missense variant in a gene for which primarily truncating variants are known to cause disease
BP5 Supporting	Found in case with an alternate molecular basis for disease
Summary	1 X Strong, 2 X Supporting = Likely benign
SETX variant c.5825T>C, p.(Ile1942Thr)	
Criteria code weight	**Pathogenic evidence**
PS1 Strong	Same amino acid change as a previously established pathogenic variant regardless of nucleotide change. This variant has been previously reported as pathogenic [8]
PS3 Strong	Well-established functional studies show a deleterious effect
PM2 Supporting	Absent from control (or at extremely low frequencies if recessive) [15]
PP3 Supporting	Multiple lines of computational evidence support a deleterious effect on the gene/gene product
Summary	X Strong = Pathogenic
SETX variant c.1484T>C, p.(Leu495Pro)	
Criteria code weight	**Pathogenic evidence**
PS3 Strong	Well-established functional studies show a deleterious effect
PM2 Moderate	Absent from control (or at extremely low frequencies if recessive)
PM3 Moderate	For recessive disorders, detected in trans with a pathogenic variant.
PP3 Supporting	Multiple lines of computational evidence support a deleterious effect on the gene/gene product
PP5 Supporting	Reputable resource reports the variant, but evidence not available
Summary	1 X Strong, 2 Moderate, 2 Supporting = Pathogenic

3. Discussion

In the present study, we have reported a patient with an initial suspected diagnosis of ATX-ATM and homozygous variants in ATM in whom subsequent functional analysis was critical in establishing the diagnosis of ATX-SETX, highlighting that ATX-SETX should be considered in the differential diagnosis of ATX-ATM.

Although sequential single gene sequencing was performed in this patient, first seen some years ago, it is widely accepted that NGS with either whole exome or genome sequencing is a more efficient diagnostic approach [1].

Advances in genetic technology have significantly improved diagnostic yield in rare diseases; however, these improvements have led to an increase in VUS [1]. VUS represent major challenges for diagnosis, management, and genetic counselling. A large study assessing utility of multigene testing identified VUS in 53% of individuals; only 0.7% of these variants were reclassified as clinically significant [11]. However, as the literature and our understanding of variants expand, it is expected that more variants are likely to be reclassified. With the increase in genetic tests being sent from general neurology clinics, access to a neurogenetics multidisciplinary meeting or discussion with local genetics laboratories is important. About 40% of ATM variants in breast cancer are VUS [12]. When ATM sequencing in an ataxia patient demonstrates VUS, functional studies to measure radiosensitivity, ATM protein level and ATM kinase activity/signalling should be performed. Functional and cytogenetic studies and an alternate molecular diagnosis in our patient support that ATM c.1010G>A is not pathogenic, also indicated by other authors, and should be reclassified as likely benign [6]. This has important clinical implications for the patient and her family, as her breast cancer is likely sporadic. Furthermore, the reclassification of this variant has implications for carriers of this variant, as they are not at increased cancer risk.

Pathogenic recessive variants in SETX, encoding an enzyme thought to function as a helicase in DNA transcription and RNA processing, cause ATX-SETX [12]. Our patient had a known pathogenic variant c.5825T>C but a younger age at symptom onset than reported previously with this variant. She also had peripheral neuropathy, not previously documented with this variant, but a common finding in patients with ATX-SETX [8]. This may be due to the effect of the second variant c.1484T>C. Functional studies demonstrated reduced level of senataxin, supporting pathogenicity.

In summary, this case highlights the challenges with interpretation of variants of uncertain significance. The ease of access and widespread use of genomic sequencing will increase the return of VUS; interpretation should be cautious based on careful clinical phenotyping, easily accessible pre-existing databases (*e.g.* ClinVar) [6] and consideration of additional diagnostic steps, including familial segregation and functional studies. There are limitations to *in silico* algorithms and where possible, *in vitro* functional analysis can help discriminate between pathogenic and non-pathogenic genetic variants.

Statements

Author contribution statement. PJM, PBM, AG, MT and SMM were involved in the conception, acquisition, analysis, and interpretation of data, drafting and revising of the manuscript. KS, LYS, PB, HD and RAW were involved in the acquisition of the data and analysis, interpretation and revising of the manuscript.

Ethics statement. The authors confirm that this work complies to the journal's guidelines on issues involved in ethical publication, which state that written informed consent was obtained from individual participants involved in case studies or through a surrogate where appropriate.

Declaration of interest. The authors declare that they have no conflict of interest.

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Supplementary material

Supplementary Video 1. Eye movements. This video demonstrates tortuous conjunctival vessels and telangiectasia, interrupted pursuit movements, gaze-evoked nystagmus and hypometric saccades.
The supplementary material is available at :
https://doi.org/10.5281/zenodo.8308340

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