CLINICAL AND GENETIC CHARACTERIZATION OF HEREDITARY ATAXIAS

PH.D. THESIS

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SZEGED
2021

Publications directly related to the thesis

I. **Szpisjak L**, Obal I, Engelhardt JI, Vecsei L, Klivenyi P (2016) A novel SETX gene mutation producing ataxia with oculomotor apraxia type 2. Acta Neurol Belg 116:405-407

II. **Szpisjak L**, Zsindely N, Engelhardt JI, Vecsei L, Kovacs GG, Klivenyi P (2017) Novel AARS2 gene mutation producing leukodystrophy: a case report. J Hum Genet 62:329-333 (**IF: 2.942**)

III. Zadori D, **Szpisjak L**, Madar L, Varga VE, Csanyi B, Bencsik K, Balogh I, Harangi M, Kereszty E, Vecsei L, Klivenyi P (2017) Different phenotypes in identical twins with cerebrotendinous xanthomatosis: case series. Neurol Sci 38:481-483 (**IF: 2.285**)

IV. **Szpisjak L**, Nemeth VL, Szepfalusi N, Zadori D, Maroti Z, Kalmar T, Vecsei L, Klivenyi P (2017) Neurocognitive characterization of an SCA28 family caused by a novel AFG3L2 gene mutation. Cerebellum 16:979-985 (**IF: 3.199**)

V. Zadori D, **Szpisjak L**, Nemeth IB, Reisz Z, Kovacs GG, Szepfalusi N, Nemeth VL, Maroti Z, Toth-Molnar E, Olah J, Vecsei L, Klivenyi P, Kalmar T (2020) Predominant neurological phenotype in a Hungarian family with two novel mutations in the XPA gene – case series. Neurol Sci 41:125-129 (**IF: 3.307**)

VI. **Szpisjak L**, Szaraz G, Salamon A, Nemeth VL, Szepfalusi N, Veres G, Kincses B, Maroti Z, Kalmar T, Rydzanicz M, Ploski R, Klivenyi P, Zadori D (2021) Eye-tracking-aided characterization of saccades and antisaccades in *SYNE1* ataxia patients – a pilot study. BMC Neurosci 22:7, pp. 1-12 (**IF: 3.288**)

VII. **Szpisjak L**, Szaraz G, Salamon A, Nemeth VL, Szepfalusi N, Veres G, Kincses B, Maroti Z, Kalmar T, Rydzanicz M, Ploski R, Klivenyi P, Zadori D (2021) Correction to: Eye-tracking-aided characterization of saccades and antisaccades in *SYNE1* ataxia patients – a pilot study. BMC Neurosci 22:16, pp. 1-2

Total impact factor of papers directly related to the thesis: 15.021

Publications not directly related to the thesis

I. Veres G, **Szpisjak L**, Bajtai A, Siska A, Klivenyi P, Ilisz I, Foldesi I, Vecsei L, Zadori D (2017) The establishment of tocopherol reference intervals for Hungarian adult population using a validated HPLC method. Biomed Chromatogr 31, pp. 1-8 (**IF: 1.688**)

II. **Szpisjak L**, Zadori D, Klivenyi P, Vecsei L (2019) Clinical characteristics and possible drug targets in autosomal dominant spinocerebellar ataxias. CNS Neurol Disord Drug Targets 18:279-293

III. Salamon A, Zadori D, **Szpisjak L**, Klivenyi P, Vecsei L (2019) Opicapone for the treatment of Parkinson's disease: an update. Expert Opin Pharmacother 20:2201-2207 (**IF: 2.878**)

IV. Aranyi Zs, **Szpisjak L**, Szoke K (2019) Multiphasic presentation of neuralgic amyotrophy associated with hepatitis E virus infection. Muscle Nerve 61:108-110 (**IF: 2.505**)

V. Salamon A, Zadori D, **Szpisjak L**, Klivenyi P, Vecsei L (2020) Neuroprotection in Parkinson's disease: facts and hopes. J Neural Transm (Vienna) 127:821-829 (**IF: 3.575**)

VI. **Szpisjak L**, Salamon A, Zadori D, Klivenyi P, Vecsei L (2020) Selecting dopamine depleters for hyperkinetic movement disorders: how do we choose? Expert Opin Pharmacother 21:1-4

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VIII. Salamon A, Zadori D, **Szpisjak L**, Klivenyi P, Vecsei L (2020) Fixed-dose combination therapy for Parkinson's disease with a spotlight on entacapone in the past 20 years: a reduced pill burden and a simplified dosing regime. Expert Opin Pharmacother 21:2265-2278 (**IF: 3.889**)

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Total impact factor of papers not directly related to the thesis: 18.659
Cumulative impact factor: 33.680

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List of abbreviations

ACE	Addenbrooke's Cognitive Examination
AFP	alpha-fetoprotein
AD	autosomal dominant
ADCA	autosomal dominant cerebellar ataxia
ALS	amyotrophic lateral sclerosis
AOA2	ataxia with oculomotor apraxia type 2
AR	autosomal recessive
ARCA	autosomal recessive cerebellar ataxia
BDI	Beck Depression Inventory
BDST	Backward Digit Span Task
BVMT-R	Brief Visuospatial Memory Test-Revised
CA	cerebellar ataxia
CBTT	Corsi Block-Tapping Test
CCAS	cerebellar cognitive and affective syndrome
CES	clinical exome sequencing
CK	creatine-kinase
CSF	cerebrospinal fluid
CTX	cerebrotendinous xanthomatosis
DNA	deoxyribonucleic acid
DRPLA	dentatorubro-pallidoluysian atrophy
DST	Digit Span Task
ENG	electroneurography
ExAC	The Exome Aggregation Consortium
FA	Friedreich's ataxia
gnomAD	The Genome Aggregation Database

HC	healthy control
HRSD	The Hamilton Rating Scale for Depression
LST	Listening Span Task
MAF	minor allele frequency
MDS-UPDRS	Movement Disorder Society Unified Parkinson's Disease Rating Scale
MMSE	Mini-Mental State Examination
MRI	magnetic resonance imaging
MSA-C	multiple system atrophy cerebellar type
NART	National Adult Reading Test
NGS	next-generation sequencing
OMA	oculomotor apraxia
OMIM	Online Mendelian Inheritance in Man
PolyQ	polyglutamine
RBMT	Rivermead Behavioural Memory Test
RCFT	Rey Complex Figure Test
RNA	ribonucleic acid
SARA	Scale for the Assessment and Rating of Ataxia
SCA	spinocerebellar ataxia
SCAR	spinocerebellar ataxia, autosomal recessive
STAI	The State-Trait Anxiety Inventory
TMT	Trail Making Test
WCST	Wisconsin Card Sorting Test
WES	whole exome sequencing
XP	xeroderma pigmentosum
XPA	xeroderma pigmentosum type A

SUMMARY

Hereditary cerebellar ataxias (CA) are genetically heterogenous group of neurodegenerative diseases, the clinical hallmarks of which are gait and limb ataxia and dysarthria. In the last decades numerous novel disorders and their genetic background have been discovered, whereas the clinical features of some previously reported entities have broadened. The aim of the current research was to characterize and to describe some new aspects of the clinical phenotype of patients with hereditary ataxias and to identify the underlying genetic abnormalities.

Our approach was started with a detailed patient history with some disease-specific aspects and followed by a thorough neurological physical examination to recognize cerebellar ataxia as a dominating movement disorder. After that, an extensive differential diagnostic process was performed with several examinations, including laboratory, neuroimaging and neurophysiological methods to exclude the possible acquired causes of CAs. After ruling out the secondary etiologies, different genetic tests were applied to reveal the causative genetic abnormalities of hereditary CAs.

According to this procedure, novel pathogenic mutations were found in the following genes: *SETX, AARS2, XPA, AFG3L2* and *SYNE1*. The rarity and the difficulty of recognition of these disorders are well illustrated by the fact that most of them were the first identified patients or families in Hungary. Besides the new genetic variants, exhaustive neurocognitive assessment was performed in the *SYNE1* ataxia subjects and spinocerebellar ataxia (SCA) type 28 and xeroderma pigmentosum type A (XPA) families as well. The disturbances revealed by the neuropsychological examination were very similar to that found in other hereditary CAs and seems to be a part of cerebellar cognitive and affective syndrome. Additionally, device-aided eye-tracking investigation was carried out in *SYNE1* ataxia subjects to characterize the parameters of their saccades and antisaccades and to compare them with the same eye movements of Friedreich's ataxia subjects and healthy controls. This assessment demonstrated the high frequency of hypometric saccades, decreased saccadic velocity and large amount of incorrectly performed antisaccades, the latter demonstrating inverse relationship with working memory test results.

I. INTRODUCTION

The term ataxia means a pathological condition, characterized by uncoordinated movements. In neurology it denotes a clinical syndrome of imbalance and incoordination, however, it is also used to specify a group of neurodegenerative disorders with the key feature of progressive ataxia (Klockgether 2010). Ataxia as a clinical syndrome can originate from the disturbance of cerebellar, somatosensory and vestibular systems or the combination of them (Chhetri et al. 2010). Proprioception within the somatosensory system, together with the vestibular information, projects to the cerebellum, where they are processed and integrated to maintain proper balance and coordination. Anatomically, the cerebellum can be divided into vermis and two hemispheres, whereas functionally, three components can be separated: the vestibulocerebellum, spinocerebellum and cerebrocerebellum (Baehr et al. 2005). The vestibulocerebellum or archicerebellum is the oldest part from a phylogenetical point of view; its main afferent input comes from the vestibular organ and it controls the balance of the body. The spinocerebellum receives its main inputs from the spinocerebellar tracts, and it regulates the stance and gait. The cerebrocerebellum or neocerebellum is phylogenetically the youngest division, which obtains its main afferent information from the motor cortex and modulates the fine movements of the limbs (Baehr et al. 2005). Moreover, some parts of the cerebellum, especially the vestibulocerebellum, oculomotor vermis (lobules VI and VII) and the fastigial nuclei, are the chief regulators of eye movements (Beh et al. 2014). The most frequent cerebellar symptoms are gaze-evoked nystagmus, saccadic smooth pursuit, dysmetric saccades, skew deviation, dysarthric speech, limb dysmetria and ataxia, intention tremor, dysdiadochokinesia, rebound phenomenon, truncal ataxia, hypotonia and gait ataxia (Bodranghien et al. 2015). Besides the motor signs, cerebellar lesions can cause cognitive and affective disturbances, as well: In 1998, Schmahmann and Sherman published patients with cerebellar damage-induced impairment of executive functions, visuo-spatial organization and memory, language skill deficits and affective changes, including flattening of affect and disinhibited or inappropriate behaviour. This clinical observation was the basis of the entity of cerebellar cognitive affective syndrome (CCAS) or Schmahmann syndrome (Schmahmann et al. 1998).

In ataxic disorders the most common complaints are imbalance, gait disturbance, dizziness, and incoordination of purposeful movements, and slurred speech. The most important aspects

of the medical history involve the age at ataxia onset, rate of progression, co-morbidities, medications, toxic agents, and family history. In clinical practice, following the identification of cerebellar ataxia (CA), the next step is the exploration of etiological factors. First of all, the secondary or acquired causes are investigated. Acquired ataxias are characterized by acute or subacute onset and can be originated from vascular, autoimmune, infectious, toxic, malignant, vitamin deficiency or endocrine causes (Nachbauer et al. 2015). Therefore, the initial work-up including brain MRI, routine laboratory investigations, serum folic acid, vitamin B12 and E levels, antigliadin and anti-tissue transglutaminase antibodies, thyroid and parathyroid function tests, cerebrospinal fluid examinations and a detailed anamnestic survey of cerebellar toxins, aims at the exploration of these etiologies (Nachbauer et al. 2015). Following the exclusion of secondary causes, hereditary and non-hereditary degenerative disorders should be considered as the cause of CA (van de Warrenburg et al. 2014), where the disease course is usually slowly progressive. Regarding the age at onset, early-onset CA is defined when symptoms begin before the age of 25 years, while in adult-onset CA, the complaints appear after 40 years of age (Brandsma et al. 2019, Giordiano et al. 2017). Sporadic non-hereditary CAs are adult-onset disorders. The most common disease of this subgroup is multiple system atrophy cerebellar type (MSA-C) with the core clinical feature of autonomic dysfunction (Gilman et al. 2008).

Hereditary CAs can be classified according to a lot of aspects as well, however, the most relevant is the mode of inheritance, based on which, four major types can be distinguished: autosomal recessive (AR), autosomal dominant (AD), X-linked and mitochondrial ataxias. The global prevalence of autosomal dominant cerebellar ataxias (ADCAs) is 0.0-5.6 per 100.000 and the prevalence range for autosomal recessive cerebellar ataxias (ARCAs) is 0.0-7.2 per 100.000, whereas there are no clear epidemiological data of X-linked and mitochondrial CAs, yet (Ruano et al. 2014).

ADCAs, also called as spinocerebellar ataxias (SCAs), are a heterogenous group of hereditary disorders characterized by the cerebellar symptoms as their main clinical feature (Scott et al. 2020). The first classification was proposed by Anita Harding in 1982. She divided SCAs into 4 groups: ADCA type 1, featured by the presence of cerebellar and extracerebellar symptoms, ADCA type 2, characterized by cerebellar signs plus pigmentary retinal degeneration, ADCA type 3, which was a pure cerebellar ataxia, and ADCA type 4 with myoclonus and deafness

(Harding 1982). Due to the discovery of the genetic background in a lot of SCAs, the first classification was replaced by a genetic-based differentiation. Currently, more than 40 genetically distinct subtypes of SCA are known (Klockgether et al. 2019). The most common genetic variation is the CAG repeat expansion in the coding region of the gene, which encodes polyglutamine (polyQ) in the corresponding protein (Orr et al. 1993, Kawaguchi et al. 1994). PolyQ SCAs include SCA1, 2, 3, 6, 7, 17 and dentatorubro-pallidoluysian atrophy (DRPLA) (Sun et al 2016). The polyQ SCAs are the most frequent and the earliest discovered SCAs, typically characterized by a progressive disease course. A correlation exists between the number of CAG repeats and the severity of the disorder: the larger the CAG repeat number, the more severe the clinical phenotype and the earlier the age at onset. Anticipation has been observed in this group of neurodegenerative disorders due to the instability of the expanded repeat sequence during meiosis. The pathomechanism of these subtypes is the most extensively explored because of the identical genetic abnormality and their frequent occurrence in SCAs. In polyQ SCAs, similar to the other polyglutamine disorders, the expansion of glutamine causes altered protein conformation and aggregation, resulting in a reduction in the physiological function of the protein and consequential toxic effects, which eventually lead to increased neuronal vulnerability and cell death (Klement et al. 1999). SCA3, also known as Machado-Joseph disease or Azorean ataxia, is the most common autosomal dominantly inherited ataxia worldwide (Ruano et al. 2014). Besides the polyQ group, disease-causing repeat expansions have been identified in non-coding regions of the gene in some SCAs (SCA8, 10, 12, 31, 36, 37), similar to Friedreich's ataxia (FA), which is the most common autosomal recessively inherited CA (Sun et al. 2016, Campuzano et al. 1996). Moreover, conventional mutations (point mutations, deletions, insertions) can cause certain types of SCA, including SCA5, 11, 13, 14, 15/16, 19/22, 21, 23, 26-29, 34, 35, 38, 40-48, whereas in some forms of SCA, including SCA4, 18, 20, 25, 30 and 32, only the genetic loci, but not the responsible gene have been identified (Szpisjak et al. 2019, Denis et al. 2018).

ARCAs are clinically and genetically more heterogenous than ADCAs. Most of ARCAs are early-onset, however, a great part of this group was found to be a late-onset disease as well (Fogel et al. 2007). Similar to SCAs, the key clinical feature of ARCAs is spinocerebellar ataxia involving the cerebellum and its afferent and efferent pathways. In addition to

spinocerebellar signs, other neurological and non-neurological symptoms are more prevalent in ARCAs than in ADCAs, resulting in a complex multi-systemic phenotype of these disorders (Fogel et al. 2007). The deep phenotyping and the presence or absence of these extracerebellar and non-neurological signs give a clue to differentiation between ARCAs. The most important non-cerebellar neurological signs are spasticity, lower motoneuron involvement, oculomotor apraxia (OMA), ophthalmoplegia, vertical supranuclear gaze palsy, Babinski sign, intellectual disability and movement disorders including tremor, dystonia, myoclonus, chorea and parkinsonism. Relevant non-neurological signs are ophthalmological abnormalities like retinitis pigmentosa, cataract, red cherry macula and optic atrophy, but other symptoms might appear as well, such as hypogonadism, immunodeficiency, musculoskeletal symptoms (primarily scoliosis, pes cavus and short stature), diarrhea, cardiomyopathy, endocrinological problems, and dermatological alterations, including telangiectasias, xanthomas, radiosensitivity and frequent tumors of the skin (Fogel et al. 2007, Beaudin et al. 2017). Moreover, some laboratory biomarkers can help to distinguish between ARCAs, including serum level of tocopherol, albumin, lipoproteins, phytanic acid, cholestanol, alpha-fetoprotein (AFP), lactic acid, creatine kinase (CK), and ceruloplasmin (Renaud et al. 2017). The most important imaging signs, in addition to cerebellar atrophy, are spinal cord atrophy, brainstem atrophy, cerebellar white matter changes, T2-weighted linear hypointensities in the pons, middle cerebellar peduncle hyperintensities, and stroke-like lesions (Renaud et al. 2017). According to these extra-cerebellar symptoms, non-neurological signs, laboratory biomarkers and imaging features, several classification and diagnostic algorithms have been established (Beaudin et al. 2017, Beaudin et al. 2019). The most common disease in this group is FA, which is caused by biallelic pathogenic variants in the *FXN* gene (Campuzano et al. 1996). Approximately 96% of FA patients have expanded GAA repeat in the intron 1 of *FXN* gene, while 4% of patients are compound heterozygous for an abnormally expanded GAA repeat on one allele and another pathogenic variant on the other allele (Galea et al. 2016). According to the high prevalence of FA and its underlying specific genetic variation (intronic repeat expansion), after the exclusion of acquired causes of ataxia, all CA patients should undergo the *FXN* gene GAA repeat expansion testing.

SCA28 (OMIM 610246) was described in 2006 by Cagnoli et al. as a juvenile or young adult onset, slowly progressive ataxia with eye movement abnormalities. The causative gene,

AFG3L2 (ATPase family gene 3-like 2) was identified by Di Bella et al. in 2010. This gene encodes the four different domains containing mitochondrial zinc-dependent metalloprotease AFG3L2 protein, which together with paraplegin, is a subunit of m-AAA protease complex (ATPases associated with various cellular activities), and has a major role in the quality control of mitochondrial proteins (Cagnoli et al. 2010). Most of the discovered mutations causing SCA28 are missense, located in the proteolytic domain coding region of the gene. The most frequent clinical characteristics of SCA28 patients are gait and limb ataxia, dysarthria, nystagmus, ophthalmoparesis, ptosis, slow saccades, and pyramidal symptoms (Szpisjak et al. 2017). Interestingly, some heterozygous mutations of the ATPase domain of *AFG3L2* gene were identified as a cause of optic atrophy type 12 (OMIM 618977) (Caporali et al. 2020). Moreover, homozygous or compound heterozygous *AFG3L2* variants resulted in the spastic ataxia type 5 (OMIM 614487), which is an early-onset ARCA with spasticity, OMA, myoclonic epilepsy, and movement disorders, including dystonia, myoclonus, and chorea (Pierson et al. 2011).

Ataxia with oculomotor apraxia type 2 (AOA2) (OMIM 606002) is an early-onset, autosomal recessively inherited CA caused by mutations in the *SETX* gene (Moreira et al. 2004). The onset of the disorder is typically between 12-20 years of age (Anheim et al. 2009). The most common clinical features are cerebellar symptoms, sensorimotor neuropathy, OMA, pyramidal signs, and involuntary movements, such as head tremor, dystonia, and chorea. The most important laboratory biomarker is elevated serum AFP level and occasionally higher serum CK levels (Anheim et al. 2009). Brain magnetic resonance imaging (MRI) shows diffuse, remarkable cerebellar atrophy. *SETX* gene encodes the DNA/RNA helicase protein senataxin, which has a significant role in genome stability, degradation, and stress granule disassembly (Bennett et al. 2021). In addition to AOA2, certain heterozygous mutations of *SETX* gene cause another hereditary neurodegenerative disease, the juvenile amyotrophic lateral sclerosis (ALS) type 4 (OMIM 602433) (Chen et al. 2004).

AARS2-associated leukoencephalopathy (OMIM 615889) is a rare type of early or young adult-onset leukodystrophy, characterized by cerebellar symptoms, cognitive deterioration, psychiatric abnormalities, pyramidal signs, and occasional epilepsy and dystonia with ovarian failure in females (Dallabona et al. 2014). The typical brain MRI alterations of the disease are confluent, asymmetric white matter abnormalities sparing the U-fibers, predominantly in the

frontoparietal and periventricular regions, involving the corpus callosum, pyramidal tracts and other descending tracts, and cerebellar atrophy (Dallabona et al. 2014, Lakshmanan et al. 2017). The nuclear gene *AARS2* encodes the mitochondrial alanyl-tRNA synthase protein, which is involved in mitochondrial translation, and have three functionally important domains: catalytic, anticodon binding and editing ones. *AARS2* was first identified as the causative gene of fatal, early-onset cardiomyopathy (OMIM 614096) in 2011 (Götz et al. 2011). Besides the clinical phenotype of cardiomyopathy and leukoencephalopathy, recently published articles expanded the spectrum with retinopathy and optic atrophy in association with leukodystrophy, and some case reports described patients with ataxia, tremor, polyneuropathy, mild cognitive and psychiatric decline without leukoencephalopathy (Peragallo et al. 2018, Srivastava et al. 2019).

Cerebrotendinous xanthomatosis (CTX) (OMIM 213700) is an autosomal recessively inherited disease of metabolism (Björkhem et al. 2013). The main characteristic neurological symptoms of CTX are ataxia and other cerebellar signs, pyramidal symptoms, cognitive decline, parkinsonism, and seizures. The most prevalent extraneurological abnormalities are diarrhea, juvenile cataract, tendon xanthomas and psychiatric disturbances (Gallus et al. 2006). However, a pronounced heterogeneity of clinical signs between CTX patients is known, even in intrafamilial cases (Verrips et al. 2000). The causative gene is the *CYP27A1*, located in 2q35 and encoding the sterol 27-hydroxylase protein. This enzyme takes part in the appropriate production of bile acids from cholesterol, whereas its deficiency results in the accumulation of undesired lipid metabolites, including cholestanol, and also causes the insufficient production of chenodeoxycholic acid (Björkhem et al. 2013). Specific laboratory finding of CTX is the elevated serum cholestanol level, whereas the most common brain MRI abnormalities are diffuse cerebral and cerebellar atrophy, white matter lesions and bilateral signal hyperintensity of dentate nuclei (Gallus et al. 2006).

Xeroderma pigmentosum (XP) is a rare autosomal recessive condition with geographically variable prevalence. The estimated incidences of XP vary from 1 in 20,000 in Japan to 1 in 250,000 in the USA and only 2.3 per million per live births in Western Europe (Lehmann et al. 2011). There are several subtypes of XP marked with letters A-G and the variant form based on the different genetic background. The characteristic cutaneous abnormalities of XP are extreme sensitivity to sun exposure, early development of freckle-like lentiginous

pigmentation and increased risk for the evolution of malignant skin tumors. The ophthalmological alterations are conjunctival xerosis, corneal dying and conjunctivitis. The most frequent neurological symptoms involve cerebellar ataxia, cognitive decline, speech disturbance, sensorineural hearing loss, peripheral neuropathy and pyramidal signs. The neurological signs occur almost in all patients of the XPA subtype (OMIM 278700) (Moriwaki et al. 2017). The typical brain MRI abnormalities of XPA subjects are age-dependent, including severe, diffuse brain atrophy, decreased fractional anisotropy value in diffusion tensor imaging and reduced N-acetyl aspartate/creatine ratio in MR spectroscopy (Ueda et al. 2012).

Autosomal recessive cerebellar ataxia type 1 (ARCA1) (OMIM 610743), also known as spinocerebellar ataxia, autosomal recessive 8 (SCAR8) is a rare neurodegenerative disorder caused by biallelic mutations of the *SYNE1* gene (Synofzik et al. 2016). The gene is located in chromosome 6p25 and encodes the huge peptide Nesprin 1 (Nuclear envelope spectrin 1) comprising 8797 amino acids (Gros-Louis et al. 2007). Nesprin 1 is a member of the spectrin protein family, and its major function is linking the cell membrane to the actin cytoskeleton. ARCA1 or *SYNE1* ataxia was described first by Gros-Louis et al. in 2007, when 26 French-Canadian families from Quebec, Canada were reported with slowly progressive pure cerebellar hereditary ataxia caused by truncating mutations of the *SYNE1* gene (Gros-Louis et al. 2007). In 2016, Synofzik and Mademan et al. published 33 non-Canadian patients with *SYNE1* ataxia and revealed that the disease has more complex clinical phenotype than initially described (Synofzik et al. 2016, Mademan et al. 2016). The most frequent extracerebellar neurological signs are upper and lower motoneurone symptoms whereas non-neurological abnormalities include scoliosis, pes cavus and occasionally, respiratory dysfunction with severe manifestation (Synofzik et al. 2016, Mademan et al. 2016). Moreover, variants of the *SYNE1* gene have been associated with arthrogryposis multiplex congenita type 3 (OMIM 618484) and Emery-Dreifuss muscular dystrophy type 4 (OMIM 612998) (Attali et al. 2009, Zhang et al. 2007).

II. AIMS

The aims of the work were:

(1) To demonstrate the clinical phenotype of a patient with AOA2 caused by novel mutations in the *SETX* gene.

(2) To demonstrate the clinical features and brain histopathology of the first Hungarian subject with *AARS2*-associated leukoencephalopathy caused by a compound heterozygous state in the *AARS2* gene with the combination of a new nonsense and a known missense pathogenic mutations.

(3) To demonstrate the different phenotypes in identical twins with CTX.

(4) To characterize the cognitive abnormalities of the Hungarian SCA28 and XPA families and three *SYNE1* ataxia patients by neuropsychological tests.

(5) To characterize the saccadic and antisaccadic eye movements of the identified three *SYNE1* ataxia patients and compare them to the same parameters of FA subjects and healthy controls in addition to detailed clinical phenotyping and comprehensive neuropsychological assessment.

III. MATERIALS AND METHODS

III/1. CLINICAL EXAMINATION

Written informed consent was obtained from the patients for the publication of these studies and the research was approved Regional Human Biomedical Research Ethics Committee of the University of Szeged (44/2016). First of all, a detailed medical history was obtained from the patients, involving the age at onset of symptoms, present and past complaints, progression rate, usage of medications, toxic exposures, family history and previous examinations. In most instances, a pedigree was made, especially when more than one generation was affected. Then, an exhaustive neurological physical examination was performed, and the Scale for the Assessment and Rating of Ataxia (SARA) scores were recorded in many times. Based on the available data, a preliminary clinical decision-making process was started about the primary or secondary origin of the ataxia. To rule out acquired causes of ataxia, laboratory investigations were performed, including autoimmune panel, onconeural antibodies, thyroid, parathyroid hormone levels, and vitamin B12 and folate levels. At the same time, to confirm some hereditary ataxias, additional laboratory tests were performed, including serum levels of vitamin E, CK, albumin, lipids, lactate, AFP, iron, ferritin, immunoglobulins, ceruloplasmin, and rarely, cholestanol. Brain MRI and occasionally, other neuroimaging modalities were performed to differentiate between the possible causes of CA. Moreover, functional neurophysiological methods, including electroencephalography, electroneurography (ENG), electromyography, somatosensory evoked potential, and visual evoked potential measurements were applied as well. In some cases, routine and immunological cerebrospinal fluid (CSF) tests were carried out. Nevertheless, in addition to neurological symptoms, the exploration of non-neurological manifestations was important as well, especially the presence of cardiomyopathy, diabetes mellitus or other endocrine disturbances, scoliosis, pes cavus, short stature, skin lesions, diarrhea, and ophthalmological abnormalities, including cataract, optic atrophy, red cherry macula, retinal nerve fiber layer hypertrophy, and retinitis pigmentosa.

III/2. GENETIC ASSESSMENT

After ruling out the acquired causes of ataxia, written informed consent was obtained from the patients, and genomic DNA was extracted from peripheral blood leukocytes by standard protocol. First, according to the recent guidelines on the management of sporadic ataxias without known secondary etiology, the most common repeat expansion hereditary ataxias (SCA1, 2, 3, 6, 7 and FA) were investigated (De Silva et al. 2019). If these genetic examinations did not confirm the diagnosis, targeted gene testing or next-generation sequencing (NGS) was performed. Targeted gene examination was selected only if the clinical phenotype and the laboratory and/or neuroimaging biomarkers were very specific of a particular disease. The type of NGS was clinical exome sequencing (CES) in most of our cases, where a total of 60 ng of genomic DNA was used for library preparation and sequencing was performed with TruSight One clinical exome kit (Illumina) on Illumina MiSeq platform. The clinical exome kit covers the coding region of 4813 clinically relevant, disease-associated genes. The 150-bp long paired reads were aligned to the GRCh37.75 human reference genome by Burrows Wheel Aligner (BWA v0.7.9a) software. The variants were called by Genome Analysis Toolkit Haplotype-Caller (GATK v3.5) best practice; and annotated by SnpEff and VariantStudio softwares. Variants were filtered based on severity and frequency against public variant databases including dbSNP, ClinVar, ExAC, EVS and an in-house clinical exome database of 140 unrelated Hungarian patients. In addition to clinical exome sequencing, for one patient, whole exome sequencing (WES) was performed with SureSelectXT Human kit All Exon v7 (Agilent Technologies, Santa Clara, CA, USA) according to the manufacturer's instructions and paired-end sequenced (2×100 bp) on HiSeq 1500 (Illumina, San Diego, CA, USA). Prioritized variants were validated in the proband, in the parents of the proband, and in his brother by amplicon deep sequencing, using Nextera XT Kit (Illumina) and sequenced on HiSeq 1500 (Illumina).

III/3. NEUROPSYCHOLOGICAL ASSESSMENT

A detailed neuropsychological assessment was performed in the following types of hereditary CA patients: SCA28, XPA and *SYNE1* ataxia. The cognitive examination was performed by trained neuropsychologists.

In case of *SYNE1* ataxia subjects, the global cognitive performance was measured by Addenbrooke's Cognitive Examination (ACE) including the Mini-Mental State Examination

(MMSE) (Mathuranath et al. 2000). Executive functions were evaluated by verbal and semantic fluency tests. In addition, working memory and the ability to maintain and manipulate information were estimated by the Backward Digit Span Task (BDST) and the Listening Span Task (LST) (Isaacs et al. 1989, Daneman et al. 1984, Janacsek et al. 2009). The quality of information planning and visuo-constructional and visual organizational abilities were assessed by the Rey Complex Figure Test (RCFT).

In the SCA28 family, the following major neuropsychological functions were investigated: phonological and visuospatial immediate memory, working memory, executive functions, semantic memory, visual attention and speed of processing. First, to obtain a brief global cognitive assessment, ACE incorporating the MMSE was performed. Phonological immediate memory was measured with the Digit Span Task (DST) (Nemeth D et al. 2000). Visuospatial immediate memory was assessed with the Corsi Block-Tapping Test (CBTT) and the Brief Visuospatial Memory Test-Revised (BVMT-R) (Lezak 1995, Benedict 1997). Working memory was measured with the BDST and the LST. Letter, verb, episodic, and semantic fluency tests, and the Wisconsin Card Sorting Test (WCST) were performed as well to assess executive functions (Troyer et al. 1997, Cardebat et al. 1990, Heaton et al. 1993). Everyday memory functions, including semantic memory, were measured with subtests of the Rivermead Behavioural Memory Test (RBMT) (Wilson et al. 1989). Visual attention and task switching functions were investigated with the Trail Making Test (TMT) (Tombaugh 2004). The Hamilton Rating Scale for Depression (HRSD) was performed as well in two out of five patients.

Similar neurocognitive tests were used in the XPA cohort. However, to measure the mood of subjects, the Beck Depression Inventory (BDI) was applied instead of HRSD. The WCST was not performed, whereas the National Adult Reading Test (NART), the State-Trait Anxiety Inventory (STAI), and the Pieron Test were used to survey the estimated premorbid IQ, anxiety, and attention, respectively.

III/4. EYE-TRACKING

Three *SYNE1* ataxia, 6 FA patients, and 12 healthy controls (HC) were enrolled in the study. The assessment was performed in a well-lit room. Subjects sat in front of the monitor and their heads were fixed at a distance 60 cm from the screen. We used a Tobii TX300 eye

tracker and tasks were programmed in Psychophysics Toolbox V 3.0.12 under MatLab. Before every paradigm, a five-points calibration was performed.

Saccade task

Subjects accomplished the following visually guided saccade task: a black cross appeared at the center of the screen and 1.2–2 s later it jumped to the right or left side of the screen. The background was grey and the distances of displacement of the cross were 9.2° or 18.4° horizontally. All measurements were repeated 20 times in a pseudorandom order, this means 80 measurements per subject. The participants had to shift their gaze to the new position of the target as fast and accurately as they could. There was a break half-way through the task to prevent subjects tearing and/or tiring.

Antisaccade task

In the antisaccade task the simple antisaccade paradigm was used (Evdokimidis et al. 2006). The composition was similar to the visually guided saccade paradigm, however, the participants had to direct their gaze in the opposite direction (e.g., if the target appeared on the left side, they had to look to the right side). We explained in a detailed way the antisaccade paradigm to the patients before the task and answered their questions, highlighting that the antisaccade task needs more attention. Before the trial, all patients confirmed that they understood the task instructions. Only horizontal movements were recorded, as in the saccade task.

Data acquisition and processing

Data recording began when the target jumped to the periphery and stayed there for one second. The recording frequency was 300 Hz and both eyes were registered separately. We used a semi-automatic script to define parameters of saccades, as described in a previous study (Kincses et al. 2019). The following parameters were measured: peak velocity, latency, amplitude, gain, and duration. In the saccade task, we assessed the main sequence relationships of duration versus amplitude and peak velocity versus amplitude using the linear model (Federighi et al. 2011). Additionally, in the antisaccade paradigm, the incorrect ratio of antisaccades was also examined. A quotient was calculated as the number of incorrect antisaccades divided by the total amount.

IV. RESULTS

IV/1. AOA2 STUDY

A 28-year-old female patient was referred to our department with the signs of ataxia and impaired coordination. Her symptoms appeared 3 years earlier, at the age of 25, with imbalance, dizziness, and clumsiness of the hands. In the following period, her speech became slower and mildly slurred, whereas she developed gaze fixation difficulty, with intermittent diplopia and blurred vision. The neurological examination revealed mild overshooting saccadic pursuits, horizontal gaze fixation instability, OMA, slurred speech, slight ataxia in all four extremities and in the trunk, brisk tendon reflexes in the lower extremities, and normal sensory functions. The patient had no other diseases. Her parents and her brother did not report any neurological problems, but unfortunately, no more data was available about the family. The routine laboratory examinations, including a blood count and serum levels of CK and lipids, were normal. The serum AFP level was elevated to 21.9 ng/ml (normal < 7.0 ng/ml). The brain MRI demonstrated moderate cerebellar atrophy with normal supratentorial structures (Figure 1). Cardiological, diabetological, and fundoscopic examinations did not find any pathological abnormalities.

Genetic investigation for Friedreich's ataxia resulted in normal GAA repeat numbers (7 and 13 repeats). Considering the young age at onset, the OMA, the elevated serum AFP level, the moderate cerebellar atrophy, and the lack of oculocutaneous telangiectasias, the diagnosis of AOA2 was hypothesized. Consequently, *SETX* gene sequencing analysis was performed (Centogene AG, Rostock, Germany), which identified a novel heterozygous point mutation, c.502C>T, p.Arg168Trp in exon 6. After that, multiplex ligation-dependent probe amplification test was executed and revealed a large heterozygous *SETX* gene deletion, including exons 11–15. At the time of publication of the original study, the allele frequency of the c.502C>T missense variant in the Exome Aggregation Consortium (ExAC) database was very low (1/73,152 alleles in the European population). Currently, the allele frequency of this variant is 2/250,950 according to the Genome Aggregation Database (gnomAD) (https://gnomad.broadinstitute.org/variant/9-135211899-G-A?dataset=gnomad_r2_1) and is predicted to be deleterious by SIFT, and probably damaging by PolyPhen2 softwares. The consecutive arginine – tryptophan change caused by the missense mutation is located within a highly conserved region of the protein senataxin. We presume that the compound

heterozygous state of these mutations is responsible for the *SETX* insufficiency and the AOA2 disease. We were unable to perform the segregation analysis, because the father of the patient was dead, and her closest relatives (mother and brother) did not give their consent to genetic testing.

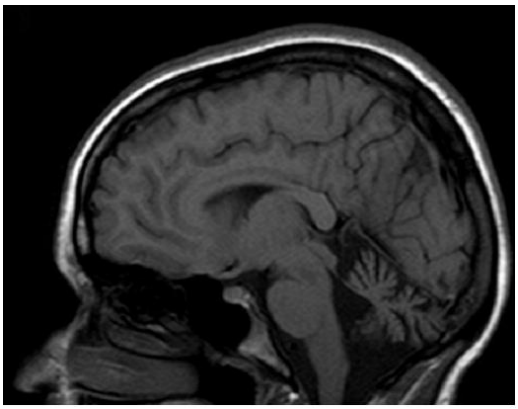

Figure 1. The sagittal T1-weighted brain MRI of the patient with ataxia with oculomotor apraxia type 2. Moderate cerebellar atrophy can be observed (Szpisjak et al. 2016). MRI: magnetic resonance imaging.

IV/2. *AARS2*-ASSOCIATED LEUKOENCEPHALOPATHY STUDY

The patient was a 29-year-old male with an uneventful perinatal period and normal childhood without serious illnesses. His grandparents, parents, and his younger brother did not report any neurological or psychiatric problem. The parents of patient noted that the behavioural changes of the subject began at the age of 18 years with the alteration of personality, mania, and paranoid delusions. Therefore, he was admitted to a psychiatric unit, where he received antipsychotic drug treatment. Some years later, movement disorders appeared, which were attributed to the side effects of the applied antipsychotic drugs. Otherwise, his intellectual development was normal with an excellent performance in primary school and in high school as well. At the age of 24 years, his cognitive impairment became obvious and a rapid and progressive intellectual decline was observed. During the following 2 years he developed acalculia, orientation problems and dysgraphia. Besides these cognitive disturbances, the neurological examination revealed horizontal and vertical nystagmus, dysarthria, dysphagia, movement disorders (including rigidity, hypo- and bradykinesia, ataxia), pyramidal tract involvement (such as Babinski sign and brisk tendon reflexes), and frontal liberation signs, (like glabellar, palmomental, and sucking reflexes and palmar grasp). Ophthalmoscopy and cardiological investigation did not reveal any abnormalities. In the following years, the patient became bedridden and fed via percutaneous endoscopic gastrostomy tube due to serious

dysphagia. The brain MRI represented a picture of leukodystrophy; extensive white matter abnormalities were noticeable, predominantly in the frontal and parietal lobes, with a relative sparing of the central region. There was a moderate cerebellar atrophy and the corpus callosum was affected as well, especially in the frontal region. Pyramidal tract involvement was visible at the level of the internal capsule, and the frontopontine fibers were selectively affected within the internal capsule and in the brainstem as well (Figure 2). Various laboratory examinations were performed to determine the precise diagnosis of this early-adulthood-onset leukodystrophy. These include a normal complete blood count, CK, and serum lactate levels. The CSF parameters, including leukocytes, protein content, and IgG index were within the normal range, and isoelectric focusing did not display any abnormalities. Polymerase chain reaction tests of the herpes simplex virus type 1 and 2 from the CSF and HIV-1 test from blood were negative. The specific laboratory examinations, including the serum levels of very long chain fatty acids and phytanic acid, the enzyme activity of arylsulfatase-A and galactocerebrosidase from leukocytes, and the blood mass spectrometry for amino acids, acylcarnitine and succinylacetone, did not demonstrate any pathological abnormalities. Moreover, the Filipin staining in cultured fibroblasts was normal as well.

Since the previous lab tests did not show any abnormalities, the possibility of Cerebral Autosomal-Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy or vanishing white matter disease emerged. Targeted genetic tests for the *NOTCH3* gene and the most common vanishing white matter disease causing gene, *EIF2B5,* were also negative. Thus, in frame of the reevaluation of the clinical phenotype and MRI features, we reviewed of the most recent literature data of leukoencephalopathy. Based on these, a new entity, the *AARS2*-associated leukoencephalopathy seemed to be the most likely diagnosis. Targeted gene sequencing of *AARS2* gene (NM_020745.3) was performed and it identified three variants: c.578T>G, c.595C>T and c.2188G>A. The c.578T>G single-nucleotide polymorphism can be considered a pathogenic variation as it causes a nonsense mutation (p.Leu193*) in exon 3 and is likely to result in messenger RNA degradation by nonsense-mediated decay. This mutation was not described earlier, and it could not be found in the ExAC database. The c.595C>T variant causes a missense mutation (p.Arg199Cys) in exon 4 of the gene and its presence was also detected in four unrelated patients with similar clinical

presentation. At the time of publication of the original study, the frequency of this allele was 14/72,800 in the European population. The c.2188G>A single-nucleotide polymorphism also resulted in a missense mutation (p.Val730Met), but it was considered a polymorphism based on its high allele frequency in control subjects (~4% in the European population according to the Exac database). The segregation analysis indicated that the pathogenic mutations are not in the same allele of the *AARS2* gene, as the father of the patient carries only the c.578T>G nonsense mutation, whereas his mother bears only the c.595C>T variant. Consequently, the patient has a compound heterozygous mutation resulting in a late onset leukodystrophy.

Before the identification of the genetic abnormality, a biopsy sampling from the frontal lobe, including the cortex and the white matter, was performed by another department. There was a lack of necrotic damage, inflammatory infiltration, neuronal changes or vascular lesions in the hematoxylin–eosin staining. In the Klüver–Barrera myelin staining, together with the immunostaining for myelin basic protein and neurofilament, we did not observe selective demyelination or axonal changes. Furthermore, there was a lack of amyloid precursor protein immunoreactive axonal bulbs. Immunostaining for CD3 (T cells), CD8 (cytotoxic T cells) and CD20 (B cells) was completely negative. There was an absence of macrophage activity (CD68) and only slight microglial reaction was noted (HLA-DR). Screening for pathological protein inclusion using immunostaining for ubiquitin and p62 did not reveal unequivocal pathological deposits (Figure 3). In summary, this biopsy did not indicate pathological alterations, in particular, either selective demyelination or axonal/myelin pathology.

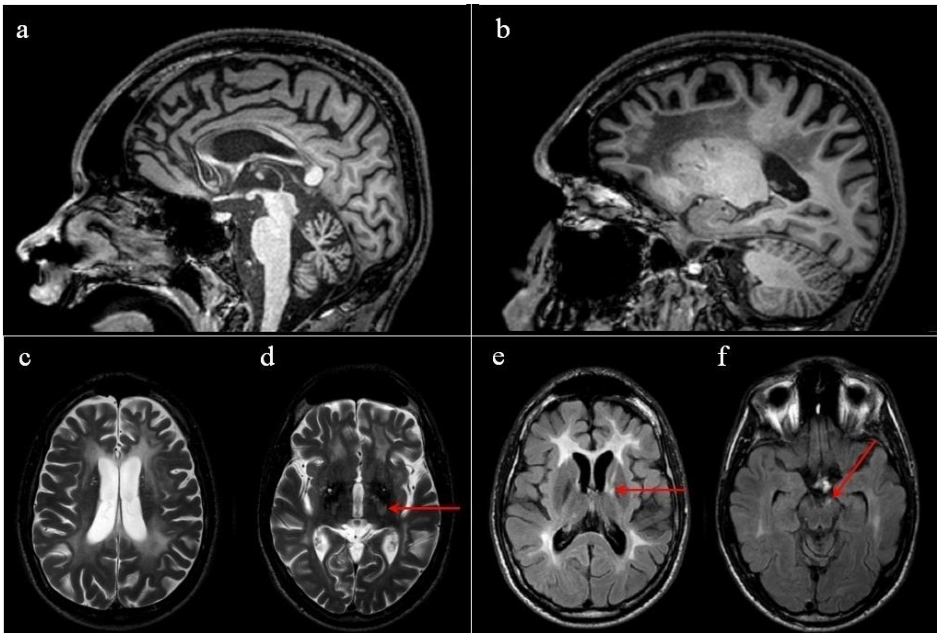

Figure 2. Brain magnetic resonance imaging of the patient with *AARS2*-associated leukoencephalopathy. **a, b)** T1-weighted, sagittal scans show corpus callosum involvement mainly in the frontal part and extended white matter abnormalities in the frontoparietal region with relative sparing of the central area. **c, d)** T2-weighted, axial scans demonstrate pronounced leukoencephalopathy. The arrow indicates the lesion of pyramidal tract in the level of internal capsule. **e, f)** Fluid-attenuated inversion recovery-weighted, axial scans: the arrows show the frontopontine tract involvement in the level of internal capsule and the cerebral peduncle (Szpisjak et al. 2017).

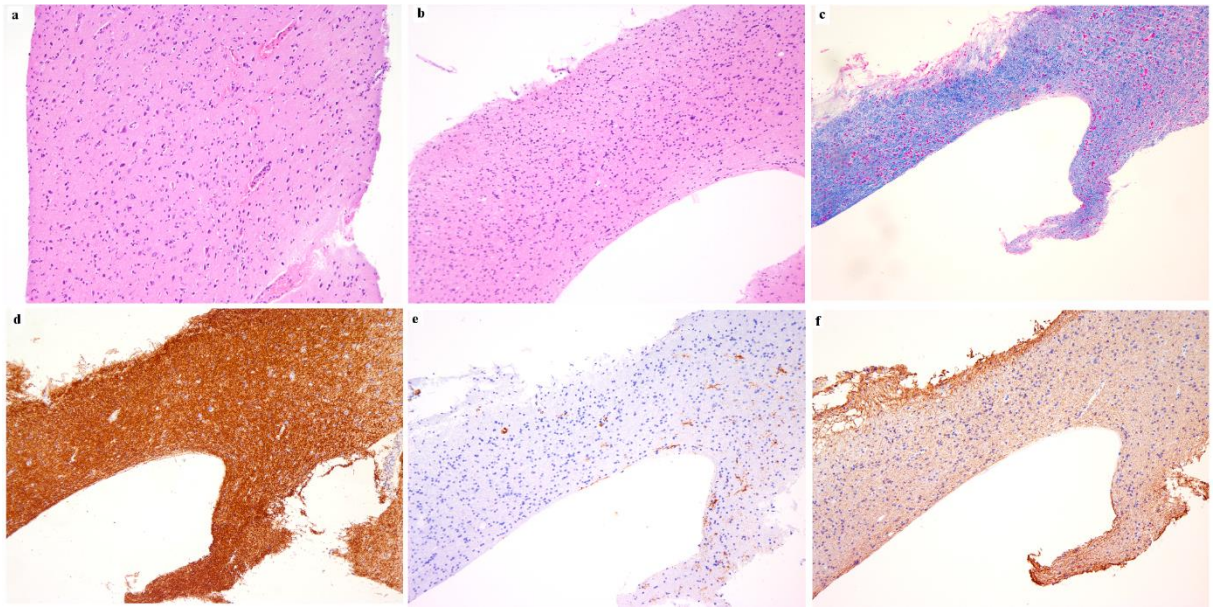

Figure 3. Representative histopathological images of the biopsy sample from the frontal lobe of the patient with *AARS2*-associated leukoencephalopathy. **a)** HE staining of the cortex

revealed preserved neurons. HE **(b)**, Klüver–Barrera **(c)**, immunostaining for myelin basic protein **(d)**, HLA-DR (microglia marker) **(e),** and SMI-31 (neurofilament) **(f)** of the white matter do not show disease-specific alterations, inflammation or demyelination (Szpisjak et al. 2017). HE: hematoxylin and eosin, HLA-DR: Human Leukocyte Antigen – DR isotype.

IV/3. CTX STUDY

The proband was a 40-year-old female patient from a twin pair who was admitted to our neurology department because of her movement disorder. The subject had cataracts and glaucoma in childhood, but her movement and speech only started to progressively deteriorate three years before her admission. Her parents mentioned memory disturbances, anxiety, and impatience. In addition to neurological and ophthalmological abnormalities, she complained of an episode of pronounced diarrhea and gastrointestinal discomfort in the year before admission. The neurological check-up revealed that the dominating movement disorder of the patient is parkinsonism, including moderate symmetric hypo- and bradykinesia, rigidity, mainly right-sided limb rest tremor, severe postural instability, freezing of gait, antecollis, hypomimia, severe dysarthria and mild seborrhea. The patient scored 75 points in the part III of Movement Disorder Society Unified Parkinson's Disease Rating Scale (MDS-UPDRS) in OFF state; whereas after levodopa treatment, it was 56 points, which denotes some levodopa response (25.3% improvement). In addition to parkinsonism, the patient had mild cerebellar ataxia and pyramidal signs, including brisk patellar reflex and ankle clonus on the right side and bilateral Babinski sign. She could not stand and walk unaided. The neuropsychological assessment demonstrated moderate cognitive impairment in light of 65/100 points in the ACE and 24/30 points in the MMSE. The brain MRI revealed T2 and FLAIR signal abnormalities in the dentate nuclei and some supratentorial white matter alterations (Figure 4a-d). The clinical features and the MRI disturbances raised the possibility of CTX, therefore, serum cholestanol measurement was performed, which was elevated as well (31 μM; normal range 2–12.6 μM). The targeted genetic testing for a disease-causing mutation in the *CYP27A1* gene revealed a known pathogenic homozygous frameshift variant in exon 4 (c.819delT, p.D273EfsTer13).

The twin pair of the proband also had juvenile cataracts and glaucoma and was suspected to have an immunological disorder; however, she did not complain any neurological problems. Nevertheless, her parents reported mild memory problems, anxiety and, impatience, but these

alterations were less expressed than the cognitive and mental changes of the proband. Despite the absence of pronounced neurological complaints, this second patient was also screened for possible signs of CTX. The examination revealed gentle sensory ataxia with a slightly broad-based gait and signs of discrete parkinsonism, including mainly left-sided hypo- and bradykinesia, rigidity on provocation in the left upper limb and moderate postural instability. She scored 8 points in the MDS-UPDRS part III in OFF state. The neuropsychological assessment revealed mild cognitive impairment in light of 72/100 points in the ACE and 27/30 points in the MMSE. The brain MRI showed very similar signal abnormalities in the dentate nuclei as it was seen in the proband (Figure 4e-f). Furthermore, the MRI revealed asymptomatic mega cisterna magna and a small tentorial meningioma (Figure 4e-h). The laboratory investigation revealed elevated serum cholestanol level (36.8 μM). The genetic testing identified the same disease-causing mutation in the *CYP27A1* gene. The considerably different phenotypes suggested the question whether the twin pair is identical or not. The following 15 short tandem repeat markers were analyzed for that purpose: D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TC11, D13S317, D16S539, D2S1338, D19S433, VWA, TPOX, D18S51, D5S818, and FGA. The DNA profile of the two subjects was perfectly the same, so they are confirmed to be identical twins. The segregation analysis demonstrated that the parents were heterozygous for the assessed mutation, and they did not show any sign or symptom of CTX.

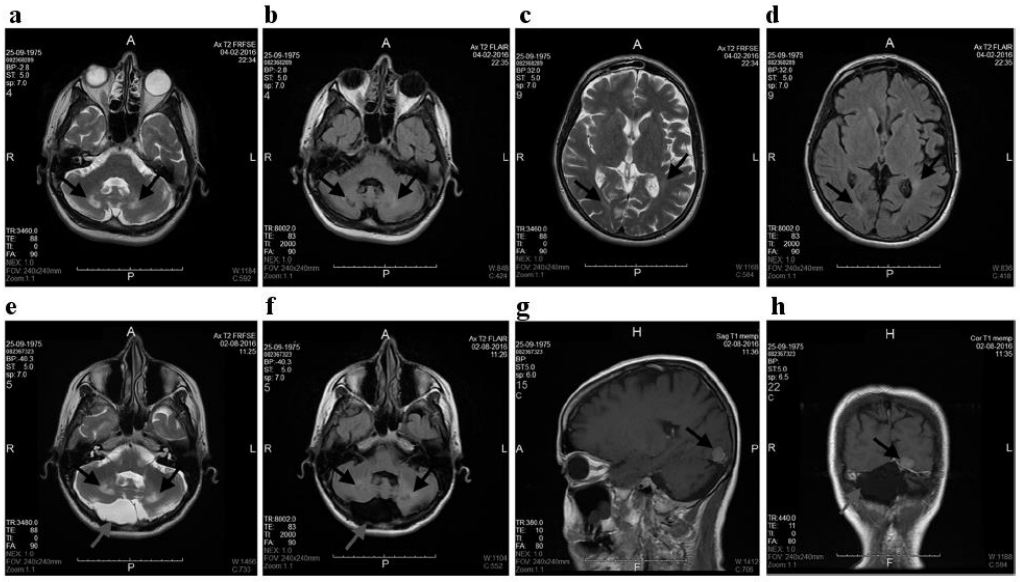

Figure 4. The brain MRI (1.5 T) images of our patients with CTX. **a-d)** The pictures of the proband. **e-h)** The images of the less-affected patient. The lesion of the dentate nuclei is prominent in both members of the identical twin pair (black arrows; T2-weighted images: a, e, FLAIR images: b, f). The proband demonstrated supratentorial white matter lesions (black arrows; T2-weighted image: c; FLAIR image: d). In the less-affected member, the MRI revealed asymptomatic mega cisterna magna (grey arrows; axial T2-weighted image: e; axial FLAIR image: f; coronal T1-weighted image: h) and a small contrast-enhancing tentorial meningioma (black arrows; sagittal T1-weighted image: g; coronal T1-weighted image: h) (Zadori et al. 2017). CTX: cerebrotendinous xanthomatosis, FLAIR: fluid-attenuated inversion recovery, MRI: magnetic resonance imaging.

IV/4. XPA STUDY

The 36-year-old Caucasian male proband (II-2 in Figure 5) was first admitted to our department for a diagnostic work-up of his unknown cognitive and movement disorder. The neurological deterioration of the patient began at his 13–14 years of age. His speech became slurred, and his cognitive functions deteriorated as well, resulting in progressive and severe learning disabilities. He completed 11 classes and later he worked in a twine factory until his 26 years of age, when his disability led to his retirement, and he became dependent on his parents. In the course of his disease repeated falls, swallowing difficulties and visual disturbances developed as well. In addition to the neurological problems, slightly exaggerated sunburn reaction was observed on the skin. Upon neurological examination eye movement alterations (exophoria, restricted eye movements in all directions with diplopia, gaze-evoked nystagmus), dysarthria, hypo-/areflexia, pathological reflexes and decreased sense of vibration were noticeable. Moreover, movement disorder with dominating ataxia and parkinsonism (bilateral dysmetria, cerebellar predominant limb ataxia more pronounced in the legs, truncal ataxia, severe postural instability, broad-based, ataxic gait, moderate, mainly left-sided bradykinesia, upper limb dystonia, mild rigidity on provocation, mild postural tremor, and occasional cortical myoclonic jerks) was detected as well. The neuropsychological investigation demonstrated severe cognitive impairment confined to two functional neuroanatomical networks, the hippocampus-dependent and that related to the prefronto-cerebellar system. The brain MRI showed remarkable generalized atrophy with slight preponderance of the parieto-occipital and cerebellar structures (Figure 6). The ENG revealed mixed type sensorimotor polyneuropathy with lower limb predominance. After the hospitalization his condition gradually worsened, and he died at the age of 39 from aspiration pneumonia. Following his death, a comprehensive postmortem neuropathological

examination was performed. The macroscopic neuropathological assessment revealed prominent generalized brain atrophy (Figure 7). The total brain weight was 815 grams, and the weight of the cerebellum-brainstem was 115 grams. The shrinkage was most prominent in the parieto-occipital region and in the infratentorial structures according to the MRI. Moreover, the hippocampus, thalamus and basal ganglia nuclei were markedly atrophic on both sides. The cross-sections of the brainstem represented an almost complete discoloration of the substantia nigra. The anterior and posterior horn of the spinal cord and the spinal nerves were proportionately atrophic. The most prominent alterations of the microscopic neuropathological examination were asymmetrical hippocampal sclerosis and Purkinje cell degeneration along with moderate loss of neurons in the substantia nigra and a scattered infiltration of CD8-positive T lymphocytes (Figures 8). With the exploration of family history of the proband, the involvement of other family members was also revealed. Similar, but less pronounced deterioration was identified in his sister (II-1) and his three brothers (II-3, 4, 5). The relevant clinical features and brain MRI characteristics are summarized in Table 1, whereas the detailed neuropsychological assessment is illustrated in Table 2. Besides a mild light sensitivity in the father (I-1), there were no major relevant symptoms in the parents; only a slight cognitive impairment was detected, which may have other explanations unrelated to XPA.

According to the cerebellar and cognitive dysfunction and the pseudo-dominant pattern of inheritance, the main causes of dominantly inherited ataxia (*ATXN1*, *ATXN2*, *ATXN3* and *TBP* genes) and cognitive dysfunction (*FMR1*, *PSEN1*, *PSEN2* and *APP* genes) were assessed first, finding no relevant alteration. Therefore, CES was performed, which identified two novel variants of the *XPA* gene: c.438_443delAGAATA, p.Gln146_Tyr148delinsHis in exon 4 and c.772_785delCGTAAGACTTGTAC, p.Arg258TyrfsTer5 in exon 6. The minor allele frequency (MAF) for the c.438_443delAGAATA variant was unknown since it was not listed in the gnomAD database, whereas the MAF for the c.772_785delCGTAAGACTTGTAC variant was 23/282,538 alleles according to the gnomAD database. Based on the American College of Medical Genetics and Genomics variant interpretation guidelines, the first mutation was classified as likely pathogenic, whereas the second as pathogenic (Richards et al. 2015). The mutation in exon 4 (p.Gln146_Tyr148delinsHis) was an in-frame deletion and affected a conserved region of the protein, which resulted in the pathologically reduced

binding of XPA to replication protein A. The variant in exon 6 (p.Arg258TyrfsTer5) eventuated a premature stop codon. The segregation analysis proved the compound heterozygosity of the patient, because the mother was heterozygous for the mutation in exon 4, whereas the father carried the exon 6 variant in heterozygous state. Both mutations were identified in all siblings of the proband as well. The genetic abnormalities found by CES were also confirmed by Sanger sequencing.

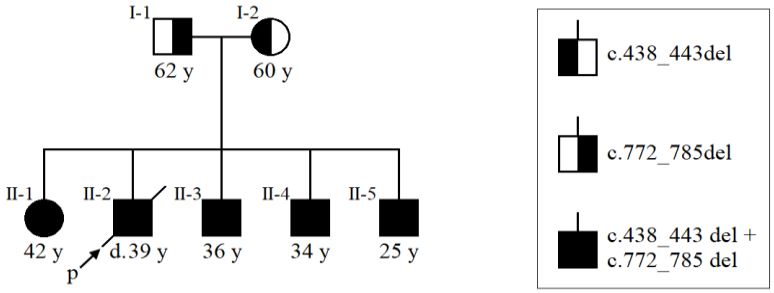

Figure 5. Pedigree of the assessed family with mutation in the *XPA* gene (Zadori et al. 2019). XPA: xeroderma pigmentosum type A.

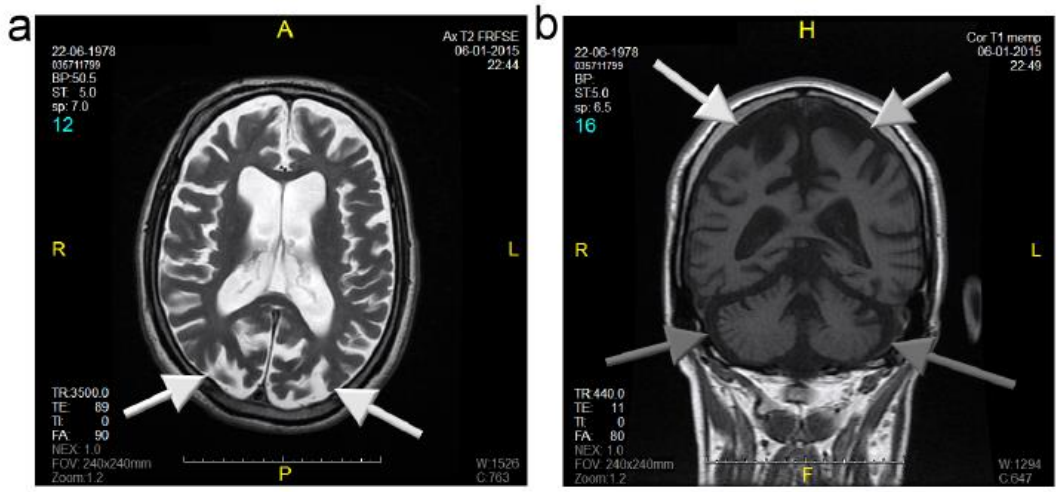

Figure 6. Brain MRI (1.5 T) of the proband with XPA demonstrates generalized brain atrophy with slight predominance at the parieto-occipital region (indicated with white arrows) and in the cerebellum (indicated with gray arrows). **a)** T2-weighted axial images. **b)** T1-weighted coronal images (Zadori et al. 2019). MRI: magnetic resonance imaging, XPA: xeroderma pigmentosum type A.

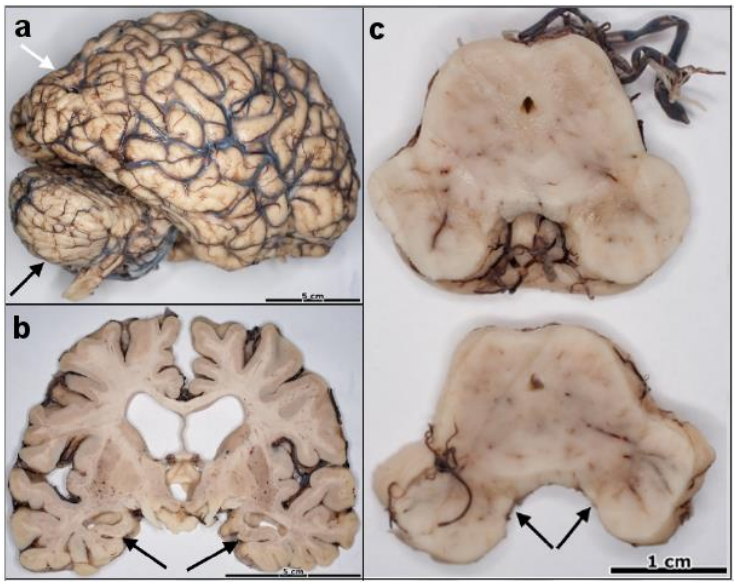

Figure 7. Macroscopic neuropathological alterations of the proband with XPA. **a)** External surface of the brain. **b)** Coronal section of the brain. **c)** Cross sections of the brain. The external examination of the hemispheres, especially that of the parieto-occipital region (a; white arrow), and the cerebellum-brainstem (a; black arrow) revealed marked atrophy. On the coronal sections a prominent thinning of the cortical ribbon could be seen, and the volume of the white matter was also decreased. The hippocampus was markedly atrophic on both sides (b; black arrows). The heads of the caudate nuclei were symmetrically flattened. The thalamus and lentiform nuclei showed mild atrophy. A remarkable ventricular dilatation and spacing in the Sylvian fissure could be seen. The cross-sections of the brainstem revealed an almost complete discoloration of the substantia nigra (c; black arrows) (Zadori et al. 2019). XPA: xeroderma pigmentosum type A.

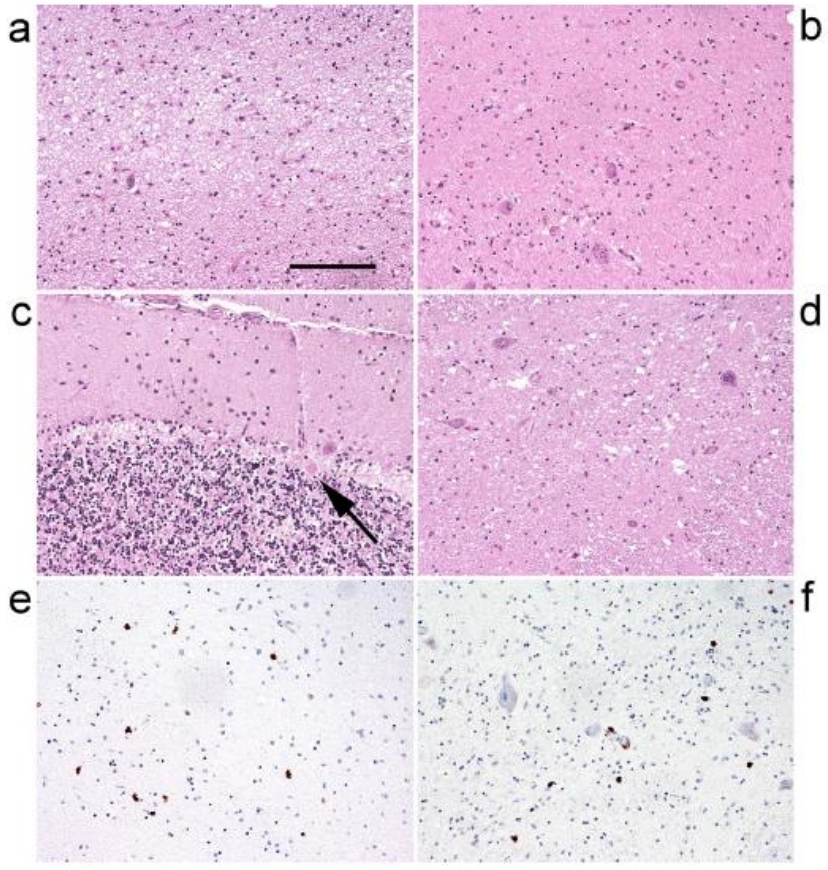

Figure 8. Microscopic *postmortem* neuropathological alterations of the proband with XPA. Formalin-fixed (*postmortem* delay of 600 hours), paraffin-embedded tissue blocks (2.5 x 2.0 cm) were applied. **a)** CA1 region, HE staining. **b)** Substantia nigra, HE staining. **c)** Cerebellum, HE staining. **d)** Anterior horn of spinal cord, HE staining. **e)** Hippocampus, immunohistochemistry, **f)** Substantia nigra, immunohistochemistry.
Histological examination revealed mild loss of neurons with edema-related spongy loosening of the neuropil in the superficial layers of cortical areas. There was a lack of ballooned neurons or eosinophilic inclusion bodies. The basal ganglia, thalamus and amygdala were also relatively well preserved. In the hippocampus, we observed prominent loss of neurons and reactive astrogliosis in the CA1 (a) and CA4 subregions. This was asymmetrical and involved mostly the left side. Further mild to moderate neuronal loss was seen in the substantia nigra (b) and severe loss was observed in the cerebellum (c), where the Purkinje cell layer showed significant depletion with Bergmann-gliosis and formation of axonal torpedoes (black arrow). Other brainstem nuclei did not show relevant neuronal loss, except for moderate gliosis in the inferior olives. The anterior horn motor neurons were moderately depleted (d). There was a lack of vascular lesions in the examined areas. Microglial activation was seen in areas with neuronal loss and additionally, scattered CD8 positive cytotoxic T cells, but no CD20 positive B cells, were also observed in the hippocampus (e), substantia nigra (f), cerebellar white matter and dentate nucleus. In further anatomical regions, mild CD8 positive cell infiltrations were seen around vessels in the white matter. Immunostaining for α-synuclein, amyloid-beta, TDP-43, phospho-TDP-43, ubiquitin and p62 did not reveal pathological protein deposits in any of these regions. The bar in (a) represents 50 micrometers for all images (Zadori et al. 2019). CA: cornu Ammonis, CD: cluster of differentiation, HE: hematoxylin and eosin, TDP: TAR DNA binding protein, XPA: xeroderma pigmentosum type A.

Table 1. The clinical and MRI characteristics of the siblings of the proband

	Patient II-1	**Patient II-3**	**Patient II-4**	**Patient II-5**
Gender	F	M	M	M
Age of neurological complaints (years)	Early 30's	Early 30's	Mid 20's	Mid 20's
Saccadic SPEM	+	+	+	+
Upward gaze paresis	+	+	+	-
Other EMA	Exophoria	-	GEN	-
Dysarthria	+	+	+	+
Tendon reflexes	Decreased/absent	Decreased/absent	Decreased/absent	Decreased
Palmomental sign	Bilateral	Bilateral	Bilateral	Unilateral
Ataxia	+	+	+	+
Cortical myoclonus	-	+	-	-
Impaired vibration sense	+	-	+	-
Cognitive decline	Moderate	Moderate	Moderate	Mild to moderate
Depression	Severe	-	-	-

Sensorineural hearing impairment	Mild	Mild	-	-
Skin lesions	Exaggerated sunburn reaction, basal cell carcinoma	Slightly exaggerated sunburn reaction	Pronounced light sensitivity, complicated scar healing	Pronounced light sensitivity
Brain MRI	Generalized atrophy, parieto-occipital and cerebellar predominance	Generalized atrophy, parieto-occipital and cerebellar predominance	Generalized atrophy, parieto-occipital and cerebellar predominance	Slight atrophy, parieto-occipital and cerebellar predominance

GEN: gaze-evoked nystagmus, EMA: eye movement abnormality, F: female, M: male, MRI: magnetic resonance imaging, SPEM: smooth pursuit eye movement (Zadori et al. 2019).

Table 2. Cognitive assessment of XPA patients and their parents

	Father	**Mother**	**Pt. II-1**	**Pt. II-2**	**Pt. II-3**	**Pt. II-4**	**Pt. II-5**
Age at examination (years)	62	60	42	39	36	34	25
Years of education	17	11	10	11	13	12	12
Estimated premorbid IQ							
NART (max. 100%)	87%	81%	82%	-	57%	63%	67%
Degree of overall impairment							
MMSE (max. 30 p)	30	28	24	19	26	27	28
ACE (max. 100 p)	89	84	63.5	35	64	70	87
Severity of impairment	-	+	++	+++	++	++	-
Verbal memory							
ACE anterograde memory (max. 7 p)	5	0	0	0	1	3	7
RBMT story delayed recall (max. 21 p)	5	6.5	0.5	-	3	0	4
Severity of impairment	+	++	+++	+++	++	++	+
Non-verbal memory							
RBMT face recognition delayed recall (max. 10 p)	10	9	10	5	8	8	9
RBMT picture recognition delayed recall (max. 20 p)	20	20	19	8	20	20	20
Severity of impairment	-	-	-	+++	-	-	-
Simple working memory							
DST	8	6	5	-	5	5	6
RBMT story immediate recall (max. 21 p)	11	7.5	2.5	-	4.5	0	6
CBTT	4	4	4	-	4	3	3
Severity of impairment	-	-	+	NA	+	+	+

Complex working memory							
LST	3	2.66	1.66	-	1.66	1.66	2
BDST	5	4	4	-	3	3	4
Severity of impairment	-	+	++	NA	++	++	+
Language							
ACE language (max. 28 p)	27	28	28	26	22	26	28
Severity of impairment	+	-	-	+	++	+	-
Attention							
Pieron test N (max. 400 p)	200	237	123	180	200	60	130
Pieron test T% (max. 100%)	98	96	84	51	81	78	96
Severity of impairment	-	-	+	+++	+	++	-
Executive functions							
Letter fluency	6.33	11	7.66	-	6.66	8.33	8.66
Semantic fluency	15	19.5	12	-	8.5	7.5	14.5
Episodic fluency	18	19	16	-	9	9	15
Verb fluency	19	16	14	-	4	7	14
TMT Part B (sec)	192	75	520	-	321	690	143
TMT Part B error	3	0	7	-	4	10	1
Severity of impairment	++	-	+++	NA	++	+++	++
Visuospatial skills							
BVMT-R (max. 12 p)	12	12	10	6	12	12	12
ACE figure drawing (max. 2 p)	2	2	2	0	2	1	1
Severity of impairment	-	-	+	+++	-	+	+
Processing speed							
TMT Part A (sec)	63	43	116	-	88	105	43
TMT Part A error	0	0	0	-	0	0	0
Severity of impairment	++	+	++	NA	+++	+++	+
Mood							
BDI (max. 63 p)	8	7	29	-	8	7	6
Severity of impairment	-	-	+++	NA	-	-	-
Anxiety							
STAI-S	32	38	47	-	30	37	41
STAI-T	37	46	49	-	36	45	35
Severity of impairment	-	-	-	NA	-	-	-

ACE: Addenbrooke's Cognitive Examination, BDI: Beck Depression Inventory, BDST: Backward Digit Span Task, BVMT-R: Brief Visuospatial Memory Test-Revised, CBTT: Corsi Block-Tapping Test, DST: Digit Span Task, LST: Listening Span Task, MMSE: Mini-Mental State Examination, NA: not available, NART: National Adult Reading Test, Pt: patient, RBMT: Rivermead Behavioural Memory Test, STAI: The State-Trait Anxiety Inventory, TMT: Trail Making Test (Zadori et al. 2019).

IV/5. SCA28 STUDY

Five affected patients from a Hungarian family, whose family tree suggested an autosomal dominant inheritance pattern, were reported to our clinic with the suspicion of hereditary ataxia (Figure 9). The first complaint of the proband (IV-5) appeared at the age of 15 years as clumsiness of the limbs, occurring exclusively after pronounced physical stress. In the following years, he developed speech disturbance, uncoordinated gait, and mild double vision. The first problems of his sister (IV-6) were writing difficulty and problems with speech when she was 28 years old. In the next generation of the family, the first symptoms of all the affected subjects were provoked by activity during physical training lessons in high school. Patient V-1 and V-3 had complaints without physical stress as well, while patient V-2 did not. The neurological assessment of the family revealed cerebellar symptoms of varying severity with dysarthria and eye movement abnormalities. Table 3 demonstrates the detailed neurological characterization of patients. Routine laboratory parameters were in the normal range, except mild elevation of serum total cholesterol levels in patients IV-5 and IV-6, and minimally increased serum CK levels in patients IV-6 and V-3. The brain MRI revealed mild to moderate cerebellar atrophy predominantly in the vermis in all patients, except patient V-2, where it was not performed (Figure 10). Otherwise, the brainstem and supratentorial structures did not demonstrate pathological abnormalities.

According to autosomal dominant inheritance, the most common polyQ SCAs were tested first in the family. However, the CAG repeats of SCA1, 2, 3, 6, and 7 were in the normal range, so in the next step, CES was performed for the proband. This NGS method identified a heterozygous missense variant c.2011G>C p.Gly671Arg in *AFG3L2* gene. This novel mutation was not found either in the 148 unrelated Hungarian controls or in dbSNP and gnomAD databases. The presence of this mutation was confirmed by targeted Sanger sequencing in the proband and in the four affected relatives as well, but was not found in a healthy sister (V-4) of patient V-3. The variant is located in exon 16 of the *AFG3L2* gene. In this position, two other pathogenic variants were detected earlier: c.2011G>A p.Gly671Arg by Cagnoli et al. and c.2011G>T p.Gly671Trp by Gorman et al. The identified amino acid change (Arg to Gly) caused by the novel missense mutation is located within a highly conserved region of the protein AFG3L2.

Table 4 demonstrates the detailed cognitive assessment of the SCA28 family. Summarily, the global examination was normal according to the ACE. However, the subjects performed slightly lower in certain aspects relative to their age and level of education. The affected cognitive functions included complex working memory, visuospatial memory, semantic memory, and executive functions.

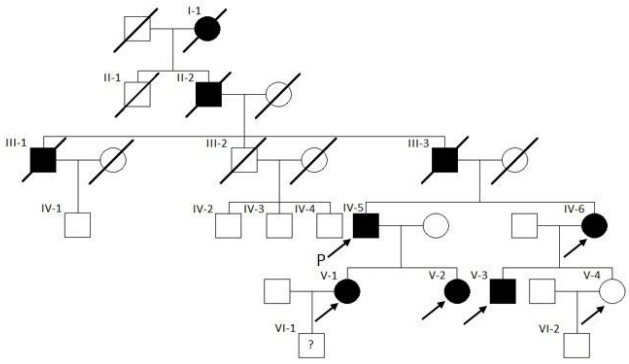

Figure 9. Pedigree of the Hungarian SCA28 family. The generations are indicated with Roman numbers while individuals in each generation with Arabic numbers. The deceased members are crossed. The patients demonstrating symptoms are in black. The proband is indicated with P, whereas the individuals seeking genetic testing with arrows (Szpisjak et al. 2017).

Table 3. Neurological characterization of the SCA28 patients

	Pt. IV-5	**Pt. IV-4**	**Pt V-3**	**Pt. V-1**	**Pt. V-2**
Age at onset (years)	15	28	14-18	14-18	14-18
Age at examination (years)	60	59	31	31	27
Gender	Male	Female	Male	Female	Female
Gait ataxia	++	++	+	+	+
Upper limb ataxia	+	+	+	+	+
Lower limb ataxia	+++	++	++	+	+
Dysarthria	++	++	+	+	+
Broken up smooth pursuit	P	P	P	P	P
GEN on horizontal testing	P	P	P	P	P
Upbeat nystagmus	P	P	-	-	-
Ophthalmoparesis	-	-	-	-	-
Slowing of saccades	-	-	-	-	-
Impaired visual acuity	P	P	-	-	-
Double vision	++	-	+	-	-
Dysphagia	++	+	-	+	-
Paresis	-	-	-	-	-
Deep tendon reflexes	brisk	normal	normal	brisk	brisk
Extensor plantar reflex	-	-	-	-	-
Hypotonia	+	+	+	++	+

Muscle atrophy	-	-	-	-	-
Chorea, myoclonus, dystonia	-	-	-	-	-
Rigidity	-	-	-	-	-
Resting tremor	-	-	-	-	-
Impaired vibration sense	-	-	-	-	-
Incontinence	-	-	-	-	-
SARA score (max. 40 p)	14	10	7.5	7.5	4.5

GEN: gaze-evoked nystagmus, Pt: patient, SARA: Scale for the Assessment and Rating of Ataxia, +: mild, ++: moderate, +++: severe, P: present, -: not present (Szpisjak et al. 2017).

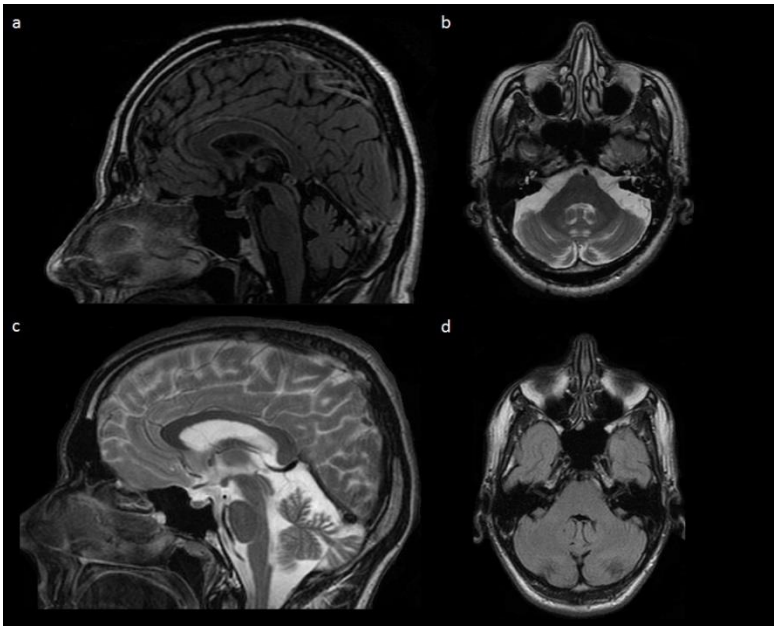

Figure 10. Brain MRI pictures of patients IV-5 and V-3 of the SCA28 family. **a)** Sagittal, FLAIR-weighted and **b)** axial, T2-weighted images of patient IV-5 demonstrate mild cerebellar atrophy, mainly in the vermis. **c)** Sagittal, T2-weighted and **d)** axial FLAIR-weighted pictures of patient V-3 indicate moderate cerebellar atrophy (Szpisjak et al .2017). FLAIR: fluid-attenuated inversion recovery, MRI: magnetic resonance imaging.

Table 4. Cognitive characterization of patients with SCA28

	Pt IV-5 (ND±SD)*	**Pt IV-4 (ND±SD)***	**Pt V-3 (ND±SD)***	**Pt V-1 (ND±SD)***	**Pt V-2 (ND±SD)***
Age at examination (years)	60	59	31	31	27
Years of education	15	16	26	17	17
ACE (max. 100 p)	91	90	96	95	96
MMSE (max. 30 p)	29	29	29	28	29
VLOM ratio in ACE	2.6	2.17	2.5	2.35	2.56
ACE orientation (max. 10 p)	9	10	10	10	10
ACE concentration (max. 8 p)	8	8	8	7	8
ACE memory (max. 35 p)	32	30	33	34	33
ACE anterograde memory (max. 7 p)	5	6	6	7	6
ACE retrograde memory (max. 4 p)	4	3	4	3	4
ACE fluency (max. 14 p)	11	10	12	12	13
ACE language (max. 28 p)	28	27	28	28	28

ACE visuospatial (max. 5 p)	3	4	5	5	4
RBMT story immediate recall (max. 21 p)	8.5	9	9.5	9	7.5
RBMT story delayed recall (max. 21 p)	6.5	10	9	8	8.5
RBMT picture recognition (max. 10 p)	10	10	10	10	10
RBMT picture recognition delayed recall (max. 20 p)	20	20	20	20	20
RBMT first and second names delayed recall (max. 1 p)	0.5	0.5	0.5	0.5	0.5
RBMT face recognition delayed recall (max. 10 p)	9	9	10	10	9
BVMT-R (max. 12 p)	11	12	12	11	12
LST	2.66 (3.11 ± 0.61)	2.66 (3.11 ± 0.61)	3.33 (3.38 ± 0.79)	3 (3.38 ± 0.79)	2.66 (3.45 ± 0.89)
DST	6	6	7	5	7
BDST	4 (5.34 ± 0.96)	4 (5.34 ± 0.96)	4 (5.88 ± 1.10)	3 (5.88 ± 1.10)	4 (5.88 ± 1.10)
CBTT	5 (6.2 ± 1.3)	5 (6.2 ± 1.3)	5 (6.2 ± 1.3)	4 (6.2 ± 1.3)	5 (6.2 ± 1.3)
TMT Part A (sec)	119 (31.32 ± 6.96)	54 (31.72±10.14)	43 (24.40 ± 8.71)	46 (24.40 ± 8.71)	53 (24.40 ± 8.71)
TMT Part A error	1	0	0	0	0
TMT Part B (sec)	415 (64.58±18.59)	126 (68.74±21.02)	62 (50.68±12.36)	79 (50.68±12.36)	81 (50.68±12.36)
TMT Part B error	6	1	0	0	0
Letter fluency	17.5 (11.02 ± 4,98)	12.5 (11.02 ± 4,98)	13.5 (16.13 ± 6.65)	12.5 (16.13 ± 6.65)	13.5 (16.13 ± 6.65)
Letter fluency perseveration	0.5 (0.025±0.045)	0 (0.025±0.045)	0 (0.02 ± 0.045)	0.5 (0.02 ± 0.045)	0 (0.02 ± 0.045)
Semantic fluency	18 (15.43 ± 4.7)	19.5 (15.43 ± 4.7)	18 (20.18 ± 6.08)	22 (20.18 ± 6.08)	21 (20.18 ± 6.08)
Semantic fluency perseveration	2 (0.02 ± 0.045)	0 (0.02 ± 0.045)	0 (0.02 ± 0.05)	0 (0.02 ± 0.05)	0.5 (0.02 ± 0.05)
Episodic fluency	22 (18.52 ± 6.34)	19 (18.52 ± 6.34)	18 (23.28 ± 8.03)	22 (23.28 ± 8.03)	19 (23.28 ± 8.03)
Episodic fluency perseveration	0.5 (0.02 ± 0.04)	0 (0.02 ± 0.04)	0 (0.01 ± 0.02)	0 (0.01 ± 0.02)	0 (0.01 ± 0.02)
Verb fluency	19	19	18	25	15
WCST Experienced categories (mean; max. 11 p)	6	6	8	8	8
WCST Completed categories (mean; max. 10 p)	5	5	7	7	7
WCST Number of tries before finding the rule (mean; min. 2 p)	5	4.25	3.67	3.33	3.17
Perseveration during search for the rule (mean; min. 0 p)	3.5	1.75	1.5	1.5	1.5
WCST Total error during known rule (mean)	5.75 (0.57 ± 1.10)	8.75 (0.57 ± 1.10)	0.33 (0.57 ± 1.10)	3 (0.57 ± 1.10)	1.33 (0.57 ± 1.10)
WCST Perseveration during known rule (mean; min. 0 p)	3.25	5.25	0.33	1.67	0.67

WCST Reaction time (median of means in msec)	5031.75	3114.25	1545.25	1533.5	1833.42
HRSD	-	11	6	-	-

ACE: Addenbrooke's Cognitive Examination, MMSE: Mini-Mental State Examination, VLOM: verbal fluency + language/orientation + memory, RBMT: Rivermead Behavioural Memory Test, BVMT-R: Brief Visuospatial Memory Test-Revised, LST: Listening Span Task, DST: Digit Span Task, BDST: Backward Digit Span Task, CBTT: Corsi Block-Tapping Test, ND: normative data, p: point(s), Pt: patient, TMT: Trail Making Test, WCST: Wisconsin Card Sorting Test, HRSD: The Hamilton Rating Scale for Depression. *Normative data are presented where available (Szpisjak et al. 2017).

IV/6. *SYNE1* ATAXIA STUDY

The AT-04 subject was referred to our department because of progressing gait disorder. He was the second child of Hungarian, non-consanguineous parents, without neurological disease in his family. The first complaint of the patient was gait disorder and delayed puberty at the age of 15 years. Later, slurred speech appeared as well, and his imbalance progressed. The neurological assessment revealed gaze-evoked horizontal nystagmus, cerebellar dysarthria, bilateral Babinski sign, gait ataxia and severe lower limb ataxia and mild numbness in the upper extremities. Occasionally, stimulus sensitive myoclonic jerks could also be observed. He had strabism and myopia with negative fundoscopy. ENG demonstrated mild axonal sensory polyneuropathy. Laboratory examination did not find pathological abnormalities. Brain MRI was performed after 16 years of disease course and displayed moderate cerebellar atrophy with preserved brainstem and supratentorial structures (Figure 11a-b).

The repeat expansion tests of FA and the most common polyQ SCAs were negative. WES identified a compound heterozygous state in *SYNE1* gene, NM_033071.3:c.8515_8516insA, p.Met2839Asnfs*53 and NM_033071.3:c.11594_11595insG, p.Glu3866*. The c.8515_8516insA variant was located in exon 55 out of 146, and it was inherited from his mother, while c.11594_11595insG was located in exon 71, and was inherited from the father. Both variants were absent in the healthy brother of the proband. None of the frameshift variants were found in the gnomAD database and they are predicted to cause the loss of the full-length Nesprin 1 protein.

Two sisters (AT-05 and AT-06) were referred to our clinic with gait problems. The age at onset of AT-05 patient was 30 years and her first symptom was gait ataxia, whereas the first symptom of her sister (AT-06) appeared at 14 years of age, and it was gait abnormality as well. The neurological examination of both patients revealed cerebellar dysarthria and brisk

tendon reflexes with bilateral Babinski signs. Truncal ataxia was moderate in the younger patient (AT-06) and severe in the elder subject (AT-05). After eleven years of disease course, patient AT-05 could only walk with aids. Mild upper limb and moderate lower extremity incoordination developed in the younger sister, whereas her sibling had moderate superior and severe inferior limb ataxia. AT-05 patient had obesity, diabetes mellitus, hypertension, and hypercholesterolemia, but ophthalmological and cardiological assessments were normal. AT-06 patient also had the same metabolic disorders, moreover, she had an excavated foot and an ENG showing multifocal sensorimotor mixed type polyneuropathy. The brain MRI revealed moderate cerebellar and very mild cerebral cortical atrophy in both patients (Figure 11c–f). Their non-consanguineous parents did not suffer from ataxia and the younger patient has two healthy children. In AT-05 and AT-06 patients, the same homozygous NM_182961.3:c.23146-2A>G variant of the *SYNE1* gene was detected. This intronic alteration was not found in gnomAD. It causes an A>G change at the Intron 127–Exon 128 boundary resulting in an abnormal splicing variant. The presence of these mutations was verified by targeted Sanger sequencing. Segregation analysis identified this variant in the heterozygous state in both parents of the subjects.

In the eye-tracking examination, the mean age of FA patients and HC group participants was the same, and the three *SYNE1* patients were in a similar age range. The demographic and clinical data of FA and *SYNE1* patients and healthy subjects are summarized in Table 5.

Saccade task

The pooled data of leftward and rightward saccades were analyzed and displayed in Table 6. There was not any relevant difference between the three groups in saccadic durations and latencies for either the shorter (9.2°) or the longer (18.4°) saccade paradigms. The peak velocities of saccades of AT-05 and AT-06 patients were smaller than the HC subjects and FA patients. However, the peak velocities of the saccades of AT-04 patient were similar to the subjects of HC and FA groups. In the 9.2° saccade task, AT-04 patient demonstrated hypermetric saccadic eye movements, whereas the other two *SYNE1* ataxia patients showed hypometric saccades. Nevertheless, in the 18.4° saccade task *SYNE1* ataxia subjects performed smaller saccadic amplitudes and gain than the healthy controls with minimal overlap (Figure 12a). The amplitudes and gain of saccades of FA patients were in a similar range to that of the HC group. Figure 13 displays the main sequence relationships using the

linear model. The duration vs. amplitude diagram (Figure 13a) demonstrates that saccades of *SYNE1* ataxia patients are hypometric and their duration is longer than in FA or HC groups. The peak velocity vs. amplitude graph (Figure 13b) reinforces that the saccades of *SYNE1* patients are hypometric and their peak velocity is smaller than in HC or FA groups.

Antisaccade task

The pooled data of leftward and rightward antisaccades were evaluated as well (Table 7). There was no remarkable difference between the groups regarding peak velocities, latencies and durations. The incorrect ratios were higher in the *SYNE1* and FA patients than in the HC group. However, there was a mildly overlapping range in the 9.2° antisaccades within the *SYNE1* and HC subjects, whereas this was only minimally detected in the longer antisaccades (Figure. 12b).

Neuropsychological assessment

The neuropsychological assessment of FA and *SYNE1* patients are summarized in Table 8. The cognitive performance of ataxia patients was compared with the data of age- and education-matched standards in the literature (Mioshi et al. 2006, Marilyn et al. 1998, Bopp et al 2005). Global cognition was only mildly reduced in two FA patients (AT-11 and AT-20), whereas the other subjects demonstrated normal ACE and MMSE scores. The LST results showed mild abnormalities in all *SYNE1* patients and in one FA patient, whereas the BDST results were decreased more prominently in both patient groups. These disturbances indicate the impairment of working memory and in the ability to maintain and manipulate information. Surprisingly, the fluency test scores were in the normal range, only AT-04 patient demonstrated a slight deficit in the verbal fluency test. In addition, the RCFT results were equal to the standard outcomes, only AT-05 patient showed a mild impairment.

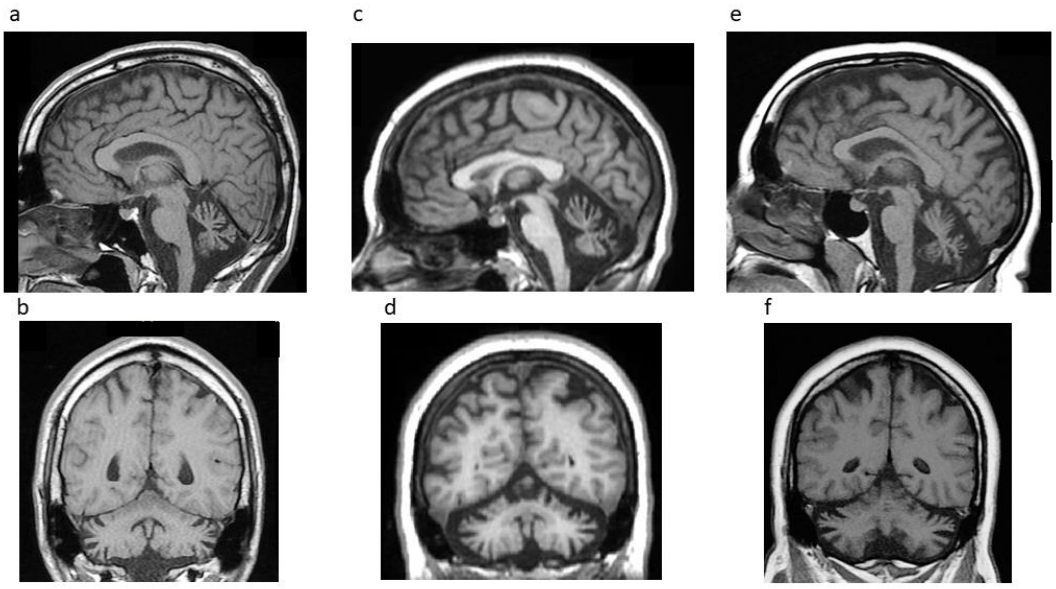

Figure 11. Brain MRI scans of *SYNE1* ataxia patients demonstrated moderate cerebellar atrophy in all subjects and mild cortical atrophy in AT-05 and AT-06 patients. **a-b)** AT-04 patient. **c-d)** AT-05 patient. **e-f)** AT-06 patient. (a, c, e: sagittal T1 weighted scans; b, d, f: coronal T1-weighted scans) (Szpisjak et al. 2021). MRI: magnetic resonance imaging.

Table 5. Demographic and clinical features of *SYNE1* and Friedreich's ataxia patients and healthy controls

Patient code/ Group name	Age (years)	Sex	Age at onset (years)	Gait ataxia	Upper limb ataxia	Lower limb ataxia	Dys-arthria	GEN	UMN	LMN	PNP	SARA
AT-04	35	M	15	+++	++	+++	++	P	P	N	mild ASN	23.5
AT-05	43	F	30	+++	++	+++	++	N	P	N	N	25
AT-06	37	F	14	++	+	+	+	N	PY	N	MSMN	12
FA group	mean: 41.5 ± 17.97 (16-60)	3M, 3F	mean: 25.83 ± 16.64 (7-49)	++5/6 +++1/6	+4/6 ++2/6	+1/6 ++4/6 +++1/6	+1/6 ++4/6 +++1/6	N 6/6	P 5/6 N 1/6	P 1/6 N 5/6	ASN 2/6 ASMN 1/6 NSP 2/6 N 1/6	mean: 16 ± 6.5 (13-30.5)
HC group (ST)	mean: 40.0 ± 10.58 (28-59)	4M, 8F										
HC group (AST)	mean: 40.25 ± 10.39 (28-59)	4M, 8F										

+: mild, ++: moderate: +++: severe, ASMN: axonal sensorimotor polyneuropathy, ASN: axonal sensory polyneuropathy, AST: antisaccade task, F: female, FA_ Friedreich's ataxia, GEN: gaze-evoked nystagmus, HC: healthy control, M: male, MSMN: mixed sensorimotor polyneuropathy, N: not present, NSP: not specified polyneuropathy, P: present, PNP: polyneuropathy, SARA: Scale for the Assessment and Rating of Ataxia, ST: saccades task, UMN: upper motor neuron involvement, (Szpisjak et al. 2021).

Table 6. Saccade parameters in *SYNE1* (AT-04-06) and Friedreich's ataxia patients and in healthy controls

	9.2° saccades					18.4° saccades				
Subjects	**PV (°/s)**	**Lat (s)**	**Amp (°)**	**Dur (s)**	**Gain**	**PV (°/s)**	**Lat (s)**	**Amp (°)**	**Dur (s)**	**Gain**
AT-04	343.18	0.16	10.27	0.069	1.117	384.95	0.19	13.87	0.079	0.754
AT-05	219.14	0.27	7.434	0.076	0.808	279.08	0.27	11.48	0.087	0.624
AT-06	215.87	0.18	7.02	0.073	0.763	280.26	0.22	15.16	0.104	0.824
Median FA (range)	316.45 (264.62-382.63)	0.20 (0.18-0.31)	9.18 (7.86-11.83)	0.071 (0.066-0.084)	0.998 (0.855-1.285)	431.13 (326.02-555.22)	0.23 (0.21-0.34)	16.49 (14.35-20.85)	0.088 (0.083-0.104)	0.896 (0.780-1.133)
Median HC (range)	269.20 (233.54-333.55)	0.18 (0.17-0.21)	8.45 (7.99-9.21)	0.069 (0.059-0.079)	0.919 (0.869-1.001)	363.93 (321.89-505.69)	0.20 (0.17-0.26)	16.75 (15.12-17.29)	0.091 (0.075-0.102)	0.911 (0.822-0.940)

Amp: amplitude, Dur: duration, FA_ Friedreich's ataxia, HC: healthy control, Lat: latency, PV: peak velocity (Szpisjak et al. 2021).

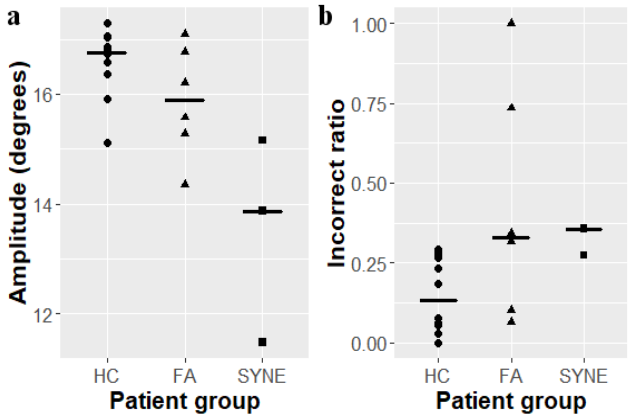

Figure 12. The most characteristic alterations in saccadic and antisaccadic paradigms in *SYNE1* ataxia patients. **a)** Saccadic amplitude of the 18.4° saccade paradigm in the different groups. **b)** Incorrect ratios of the 18.4° antisaccade task in the investigated subjects; the circles, triangles and squares denote the parameters of healthy controls (HC), Friedreich's ataxia patients (FA) and *SYNE1* subjects, respectively (Szpisjak et al. 2021).

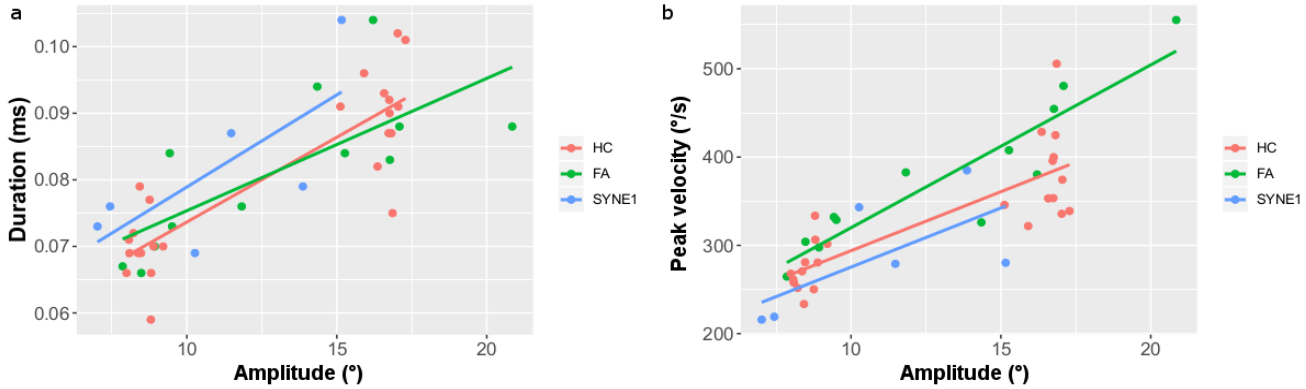

Figure 13. The main sequence relationships of saccades using the linear model. **a)** Saccadic duration versus amplitude. **b)** Saccadic peak velocity versus amplitude. The red, green and

blue dots denote the parameters of healthy controls (HC), Friedreich's ataxia patients (FA) and *SYNE1* subjects, respectively (Szpisjak et al. 2021).

Table 7. Antisaccade parameters in *SYNE1* (AT-04–06) and Friedreich's ataxia patients and in healthy controls

	9.2° antisaccades				**18.4° antisaccades**			
Subjects	**PV (°/s)**	**Lat (s)**	**Dur (s)**	**IR**	**PV (°/s)**	**Lat (s)**	**Dur (s)**	**IR**
AT-04	261.71	0.28	0.053	0.40	290.23	0.29	0.052	0.27
AT-05	232.76	0.32	0.072	0.64	280.16	0.37	0.097	0.36
AT-06	212.44	0.19	0.077	1.00	221.08	0.41	0.069	0.35
Median FA (range)	300.87 (237.22-351.78)	0.28 (0.19-0.41)	0.067 (0.059-0.072)	0.62 (0.28-0.96)	336.57 (253.63-439.47)	0.32 (0.20-0.45)	0.083 (0.062-0.103)	0.33 (0.06-1.00)
Median HC (range)	243.06 (197.67-313.18)	0.28 (0.23-0.33)	0.053 (0.046-0.075)	0.19 (0.08-0.54)	283.15 (209.63-382.10)	0.28 (0.24-0.37)	0.062 (0.049-0.088)	0.07 (0.00-0.29)

Amp: amplitude, Dur: duration, FA: Friedreich' ataxia, HC: healthy control, IR: incorrect ratio, Lat: latency, PV: peak velocity (Szpisjak et al. 2021).

Table 8. Neuropsychological assessment of *SYNE1* and Friedreich's ataxia patients

Pt code	**Age (ys)**	**Edu (ys)**	**ACE (93.7 ±4.3)**	**MMSE (28.8 ±1.3)**	**LST**	**BDST**	**Verbal fluency**	**Semantic fluency**	**RCFT copying**	**RCFT recall**
***SYNE1* patients**										
AT-04	35	14	89	29	2 * (3.38 ±0.79)	4 * (5.88 ±1.1)	10.5 * (17.61 ±5.42)	14 (17.25±3.96)	NA	NA
AT-05	43	14	93	29	2.33 * (3.38 ±0.79)	3 ** (5.88 ±1.1)	14.5 (17.61 ±5.42)	17 (17.25±3.96)	35 (31.1±3.6)	15 * (23.7 ±5.2)
AT-06	37	12	89	29	2 * (3.38± 0.79)	2 *** (5.88 ±1.1)	16 (17.61 ±5.42)	14 (17.25±3.96)	36 (31.1±3.6)	21.5 (23.7 ±5.2)
Friedreich's ataxia patients										
AT-08	23	12.5	93	30	3 (3.45 ±0.89)	4 * (5.88 ±1.1)	14.5 (16.13 ±5.65)	11 (15.84±4.51)	36 (31.1±3.6)	24 (23.7 ±5.2)
AT-11	57	16	87*	27	2.33 * (3.11 ±0.61)	3 ** (5.34 ±0.96)	12 (11.02 ±4.98)	16 (13.77±4.05)	36 (29.2±4.2)	26.5 (15.5 ±5.5)
AT-12	60	17	95	29	3 (3.11 ±0.61)	2 *** (5.34 ±0.96)	15.5 (11.02 ±4.98)	20 (13.77±4.05)	34 (29.2±4.2)	27 (15.5 ±5.5)
AT-20	16	10	88	26*	2.66 (3.33 ±0.59)	5 (5.88 ±0.96)	13.5 (13.8 3±4.31)	14 (13.44±3.52)	36 (31.1±3.6)	26 (23.7 ±5.2)
AT-21	59	14	94	30	3 (3.11 ±0.61)	5 (5.34 ±0.96)	15 (11.02 ±4.98)	14 (13.77±4.05)	32 (29.2±4.2)	17.5 (15.5 ±5.5)
AT-22	34	15	96	30	3.3 (3.38 ±0.79)	4 * (5.88 ±1.1)	16.5 (16.13 ±5.65)	21 (15.84±4.51)	34 (31.1±3.6)	21 (23.7 ±5.2)

*mild deficit, **moderate deficit, ***severe deficit (Age- and education-matched standards, and standard deviations of the literature are shown (Mioshi et al. 2006, Marilyn et al. 1998, Bopp et al 2005). Mild, moderate and severe deficits mean that the cognitive impairment of the subject is more pronounced than one, two and three standard deviations of the normal standards, respectively.) ACE: Addenbrooke's Cognitive Examination, BDST: Backward Digit Span Task, LST: Listening Span Task, MMSE: Mini-Mental State Examination, NA: not available, Pt: patient, RCFT: Rey Complex Figure Test, ys: years.
In ACE and MMSE the lower normal threshold values of the normal population are in the brackets. In LST, BDST, verbal fluency, semantic fluency, RCFT copying, and RCFT recall the age- and education-matched lower threshold values of the normal population are in the brackets (Szpisjak et al. 2021).

V. DISCUSSION

In the last decades, the group of hereditary CAs has expanded both in terms of the number of diseases and their phenotypic variability. The aim of the current research was to characterize the clinical phenotype of these patients and to identify the causative genetic background of these neurodegenerative disorders.

In the AOA2 study we found a novel missense mutation and a large deletion in the *SETX* gene in a young female subject with representative clinical features of AOA2. The missense variant, located in exon 6, affected the N-terminal domain of senataxin, whereas the extensive deletion resulted in a truncating protein structure. The clinical features of 13 other exon 6 mutation carrier AOA2 patients were more serious and their age at onset was lower (mean 12.7 years) (Tazir et al. 2009, Nanetti et al. 2013, Datta et al. 2013, Ghrooda et al. 2012). The milder phenotype of our patient suggests a residual activity of senataxin. The previously published studies reported that the phenotype was more pronounced when the mutation was missense and located outside the helicase domain (Anheim et al. 2009). The relatively mild features of our subject are possibly due to the compound heterozygosity and the short-elapsed time from the onset.

In the *AARS2*-associated leukoencephalopathy study we described a young male patient with leukodystrophy caused by compound heterozygous state of a known pathogenic missense (c.595C>T) and a novel nonsense (c.578T>G) variant of *AARS2* gene. Our subject was the eighth published patient with this disease all over the world. The features of this case was consistent with the predictions described previously that one nonsense mutation together with

the p.Arg199Cys variant cause the phenotype of leukoencephalopathy without cardiac symptoms (Euro et al 2015). Leukodystrophy is not a common finding among CA patients, therefore the characteristic MRI findings were helpful to get the diagnosis. Additionally, ovarian failure is a very common symptom in female patients of this disorder (Dallabona et al. 2014). In this study, we report the first histological data of this disease, however, as the biopsy sample involved a region that was not severely affected, histopathological examination did not reveal any disease-specific abnormality.

As with many inherited diseases, genotype-phenotype correlation is not known in CTX either. In the CTX study, an identical twin pair showed different clinical phenotype. However, the features of the proband were slightly different from typical cases reported in the literature, lacking seizures, tendon xanthomas and presenting parkinsonism as dominating movement disorder (Leitersdorf et al. 1993). On the other hand, her female twin pair demonstrated only minor disturbances. The main relevance of this study was to draw attention to the significant differences in the severity of their symptoms and to discuss the possible explanation of this diversity. One of these possibilities is that environmental factors are responsible for the differences, however, our twin pair has been continuously living together with their parents which makes this theory implausible. Another opportunity is that currently unidentified epigenetic factors may have regulatory function in the background of clinical variability.

The literature data of genotype-phenotype correlation of XPA revealed that mutations located in the exons 2, 3 and introns of the *XPA* gene are almost always characterized by severe symptoms. By comparison, the variants affecting the C-terminal region of the protein may be presumed as hypomorphic, because they can be featured by milder neurological and cutaneous abnormalities (Takahashi et al. 2010, Messaoud et al. 2012). Besides the site of the mutation, other important influencing factors of the clinical phenotype are the age of patient and the sun exposure for the skin and eye disturbances (Kondoh et al. 1995). Our study confirmed this genotype-phenotype relationship, considering that only mild-to-moderate dermatological and no pronounced ophthalmological and sensorineural hearing impairment, but prominent neurological disturbances developed with age. Despite the hypomorphic nature of the identified two novel mutations, the in-frame variant in exon 4 affects a conserved region of the peptide, inclusive of Glu 147 and Tyr 148, the deletion of which results in ineffective binding of XPA protein to replication protein A (Feltes et al. 2015). The exon 6

frame-shift mutation may be considered pathogenic as it leads to a premature stop codon. In later years, the pathogenicity of this mutation was reinforced according to gnomAD database (https://gnomad.broadinstitute.org/variant/9-100437757-AGTACAAGTCTTACG-A?dataset=gnomad_r2_1). In addition to novel mutations and the first Hungarian family of XPA, the study demonstrated a scattered infiltration of CD8+ T lymphocytes in the brain of the proband, without manifest skin lesions. In the common neurodegenerative diseases characterized by protein accumulation, the presence of CD8+ T cells is not a pathological hallmark, therefore, it raises the possibility of an immunological activation following a currently unidentified process.

In the SCA28 study, a novel disease-causing missense variant of *AFG3L2* gene was described as the genetic background of the first Hungarian SCA28 family. Compared to previous case presentations, the main difference was the absence of ptosis, ophthalmoparesis and slowing of saccades in our patients, whereas these oculomotor symptoms occurred in about half of the cases (Cagnoli et al. 2006, Di Bella et al. 2010, Cagnoli et al. 2010, Edener et al. 2010, Gorman et al. 2015, Löbbe et al. 2014, Mariotti et al. 2008, Musova et al. 2014, Qu et al. 2015, Smets et al. 2014, Svenstrup et al. 2016, Zühlke et al. 2015). One possible explanation of this difference is that these signs appear later in the disease, and the mean age of onset of these oculomotor disturbances of the earlier reported SCA28 subjects was 65.4, 57 and 55.4 years, respectively. However, only two individuals were older than 50 years amongst our patients. In addition to the genetic finding and the comparison of clinical features, detailed neurocognitive assessment was performed as well. The psychological examination detected slight disturbances in the following parts of cognition: working memory, visuospatial immediate memory, semantic memory, and executive functions. It was the first thorough neuropsychological investigation in SCA28. The deficits identified are similar in nature to those found in SCA1 (executive dysfunction), SCA2 and 3 (executive and visuospatial disabilities), reflecting the abnormalities of the cerebellar-prefrontal connection system (Fancellu et al. 2013, Braga-Neto et al. 2014, Tomlinson et al. 2014, Nagahama et al. 1996). The cognitive alterations of our SCA28 family are very similar to the most common SCAs and might be the part of the CCAS (Schmahmann et al. 1998). CCAS was reported in non-hereditary cerebellar diseases as well. The symptoms of Schmahmann's syndrome are presumably originating from the disruption of cerebellar neural pathways connecting the

cerebellum to several cortical areas, including prefrontal, superior temporal, posterior parietal and limbic, resulting in deficits of executive function, visuospatial abilities, language, and emotional regulation (Bodranghien et al. 2016).

In the *SYNE1* ataxia study, the first Hungarian *SYNE1* patients were demonstrated to be caused by novel gene mutations. In addition to the dominating cerebellar symptoms, including pronounced gait and lower extremity ataxia, mild-to-moderate upper limb ataxia and dysarthria, pyramidal signs and in two out of the three patients, polyneuropathy was observed as well. Furthermore, AT-04 subject also suffered from strabism, delayed puberty and tactile sensitive myoclonic jerks. The clinical features of our patients were not purely cerebellar, similarly the European ARCA1 subjects published by Synofzik and Mademan et al. (Synofzik et al. 2016, Mademan et al. 2016). This heterogeneity of symptoms and the other *SYNE1* associated diseases, including arthrogryposis multiplex congenita type 3 and Emery-Dreifuss muscular dystrophy type 4 suggests that *SYNE1* plays a significant role in the proper functioning of the nervous and musculoskeletal systems as well (Attali et al. 2009, Zhang et al. 2007). Despite the numerous symptoms of the discovered hereditary diseases, an obvious genotype-phenotype correlation has not been established so far (Synofzik et al. 2016).

The eye-tracking assessment demonstrated hypometric saccades in all *SYNE1* ataxia patients in the longer (18.4°) paradigm and in two out of three in the shorter (9.2°) task. Saccadic dysmetria is a common cerebellar symptom in the disease group of hereditary ataxias. This eye movement disturbance is not specific for any type of CA, however, there may be a higher frequency of hypo- or hypermetric saccades, serving as a supporting clinical feature of the disease (Moscovich et al. 2015). The hypometria of our *SYNE1* ataxia subjects in the longer paradigm was more remarkable than the well-known slight hypometria in HC and is presumably due to the involvement of cerebellar oculomotor vermis and caudal fastigial nucleus (Evdokimidis et al. 2006, Mariani et al. 2017). In addition to accuracy, velocity is a relevant feature of saccades as well, whereas the evaluation of it is often difficult by physical examination. Previously published case reports demonstrated the occurrence of saccadic slowing in ARCA1, however, these descriptions were based on physical investigation exclusively (Gros-Louis et al. 2007, Synofzik et al. 2016, Dupré et al. 2007, Noreau et al. 2013, Gama et al 2016, Kim et al. 2019). Findings of the current research confirmed these observations by fine eye-tracking method. The background of the slowness of saccades is

probably due to the impairment of brainstem, especially the dysfunction of pontine saccadic burst generator and omnipause neurones (Federighi et al. 2011). Between hereditary CAs, the low saccadic velocity was a characteristic feature of SCA2, however, at similar conditions (target eccentricities 10 and 18°), more pronounced slowing of saccades was detected in seven SCA2 patients than we observed in two of three of our *SYNE1* subjects, which denotes a more severe brainstem involvement in SCA2 (Federighi et al 2011).

The antisaccade examination demonstrated higher rates of incorrect antisaccades in both FA and *SYNE1* ataxia patients compared to HC, whereas the other parameters did not show relevant differences. The larger incorrect ratio of antisaccades raises the suspicion of cognitive impairment, especially in light of that a strong correlation was demonstrated between working memory and error rate of antisaccades (Thomas et al. 2018). Despite the preserved global cognitive performance of *SYNE1* patients, the neuropsychological assessment showed executive dysfunction with particular involvement of the working memory. The values scored by ARCA1 subjects in BDST and LST tests demonstrated inverse correlation with the error rate of antisaccade paradigm, i.e., the most prominently affected patient (AT-06) in working memory tests performed the highest incorrect ratio (Figure 14).

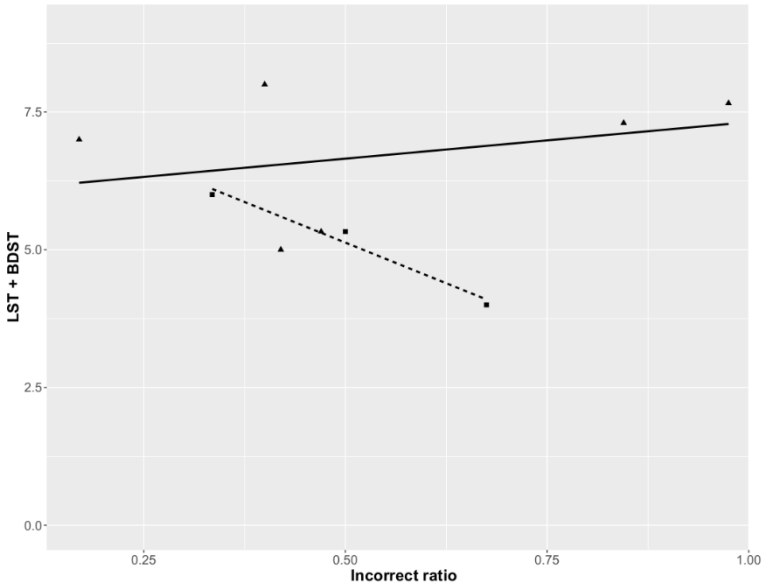

Figure 14. The delineation of the possible relationship between working memory test results and the incorrect ratio of antisaccades in ataxia patients. The axis of abscissa denotes the mean value of the incorrect ratios of 9.2 and 18.4 antisaccade tasks, whereas the axis of ordinate indicates the sum of Listening Span Task (LST) and Backward Digit Span Task (BDST) scores; the triangles and squares indicate the data of Friedreich's ataxia patients (FA) and *SYNE1* patients, and the regression line is drawn by continuous and dashed lines, respectively (Szpisjak et al. 2021).

A similar relationship was not detected in the FA group. Higher error rates of antisaccades were reported in other hereditary and idiopathic CAs as well (Mariani et al. 2017, Pretegiani

et al. 2018, Rodriguez-Labrada et al. 2014, Rivaud-Pechoux et al. 1998). In addition, detailed research of SCA2 patients confirmed that impaired antisaccade performance was associated with deficits in executive tests, including verbal fluency test and Stroop interference task (Rodriguez-Labrada et al. 2014). The recent study elucidates the importance of working memory and inhibitory control in the efficacy of antisaccades and confirms that executive dysfunction is a common cognitive alteration in hereditary CAs as a part of the CCAS (Schmahmann et al. 1998).

VI. CONCLUSIONS

The group of hereditary CAs is an expanding disease entity with a huge heterogeneity of clinical features, pathomechanism and genetic background. Despite the growing literature data of the recent years, therapeutic options are still lacking in this field. Therefore, detailed clinical investigation and exploration of the genetic abnormalities could provide essential information about the genotype-phenotype correlations and biomarkers of inherent CAs resulting in a better insight of disease pathomechanism and future target of drug design. From that reason, this thesis focused on the identification and thorough clinical characterization of patients with hereditary CA. Novel disease-causing mutations were found in the following genes: *SETX, AARS2, XPA, AFG3L2,* and *SYNE1*. Additionally, detailed neurocognitive assessments were performed in the first Hungarian *SYNE1* ataxia subjects, XPA and SCA28 families as well. Moreover, as a part of precise clinical characterization of *SYNE1* subjects, device-aided eye-tracking investigation revealed high frequency of hypometric saccades, decreased saccadic velocity and large amount of incorrectly performed antisaccades, the latter demonstrating inverse relationship with working memory test results.

VII. ACKNOWLEDGEMENTS

I would like to express my gratitude to my supervisor Péter Klivényi, M.D., Ph.D., D.Sc. (Professor and Head of the Neurology Department, University of Szeged), who introduced me to clinical neurology and raised my interest in the field of movement disorders and neurogenetics. I am grateful for his continued support in both the academic and personal areas of my life. I am especially grateful to my colleague Dénes Zádori, M.D., Ph.D. (Associate Professor at the Department of Neurology, University of Szeged) for his excellent scientific guidance and continuous support of my research activities. I would like to express my acknowledgement to László Vécsei M.D., Ph.D., D.Sc. (Former Head of the Neurology Department, University of Szeged, Member of the Hungarian Academy of Sciences) for the opportunity to work in the Department of Neurology and for his assistance in my scientific career. Furthermore, I would like to thank to all my colleagues, especially to András Salamon, M.D, Ph.D., Árpád Párdutz M.D, Ph.D., Melinda Lukács M.D., Ph.D. and Ádám Annus M.D. for their help during my work.

I would like to express my gratitude to my family, as without them this work would not have been possible. I thank my fiancée, Tímea for her aid and patience during my work. I dedicate this work to my parents who always supported and helped me in every aspect of my whole life, including my professional career.

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APPENDIX

I.

Acta Neurol Belg
DOI 10.1007/s13760-015-0569-y

LETTER TO THE EDITOR

A novel *SETX* gene mutation producing ataxia with oculomotor apraxia type 2

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Received: 18 April 2015 / Accepted: 13 November 2015

Keywords Ataxia with oculomotor apraxia type 2 · Early-onset ataxia · Novel senataxin mutation

Introduction

After Friedreich's ataxia, ataxia with oculomotor apraxia type 2 (AOA2) is the second most common autosomal recessive inherited ataxia in the European population. This disease, which has an early onset and progresses slowly, is characterized by cerebellar symptoms, oculomotor apraxia, axonal sensorimotor neuropathy, a cognitive impairment and other neurological features, including pyramidal symptoms, involuntary movements such as head tremor, dystonia and chorea [1, 2]. A typical laboratory finding is an elevated serum alpha-fetoprotein (AFP) level. In most of the cases, the brain MRI scans demonstrate marked cerebellar atrophy, mainly in the vermis. The genetic cause of AOA2 is a mutation in the *SETX* gene on chromosome 9q34, which encodes senataxin, a nuclear protein that has DNA/RNA helicase activity and possibly plays a role in DNA repair and RNA processing.

This report describes a female Caucasian patient with an AOA2 phenotype with one novel *SETX* gene mutation.

Case presentation

A 28-year-old female patient was referred to our clinic with signs of impaired coordination. The first symptoms appeared at the age of 25, with dizziness, imbalance and clumsiness of the hands. Some months later, her speech became slower and mildly slurred. She also had gaze fixation difficulty, with intermittent diplopia and blurred vision.

Her neurological examination revealed mild overshooting saccadic pursuits, horizontal gaze fixation instability, oculomotor apraxia, slurred speech, smooth ataxia in all four limbs and in the trunk, brisk tendon reflexes in the lower extremities and normal sensory functions. She had no other diseases. Her family history was unremarkable. Her parents and her brother did not report any neurological problems (Fig. 1a).

The routine laboratory findings, including a complete blood count, creatine kinase and cholesterol, were normal. The serum AFP level was elevated, at 21.9 ng/ml (normal $<$7.0 ng/ml). The brain MRI revealed moderate cerebellar atrophy with normal supratentorial structures (Fig. 2).

Cardiological, diabetological and fundoscopic examinations did not demonstrate any pathological markers. Genetic testing for Friedreich's ataxia resulted in normal GAA repeat numbers (7 and 13 repeats). The cerebellar atrophy seen in the MRI scan, the elevated AFP serum level and the lack of oculocutaneous telangiectasias led to a presumed diagnosis of AOA2. *SETX* gene sequencing analysis was therefore performed (Centogene AG, Rostock, Germany).

Gene sequencing identified a novel heterozygous point mutation in the gene: c.502C>T p.Arg168Trp in exon 6 (Fig. 1b). Multiplex ligation-dependent probe amplification testing revealed a large heterozygous deletion, including

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Published online: 25 January 2016

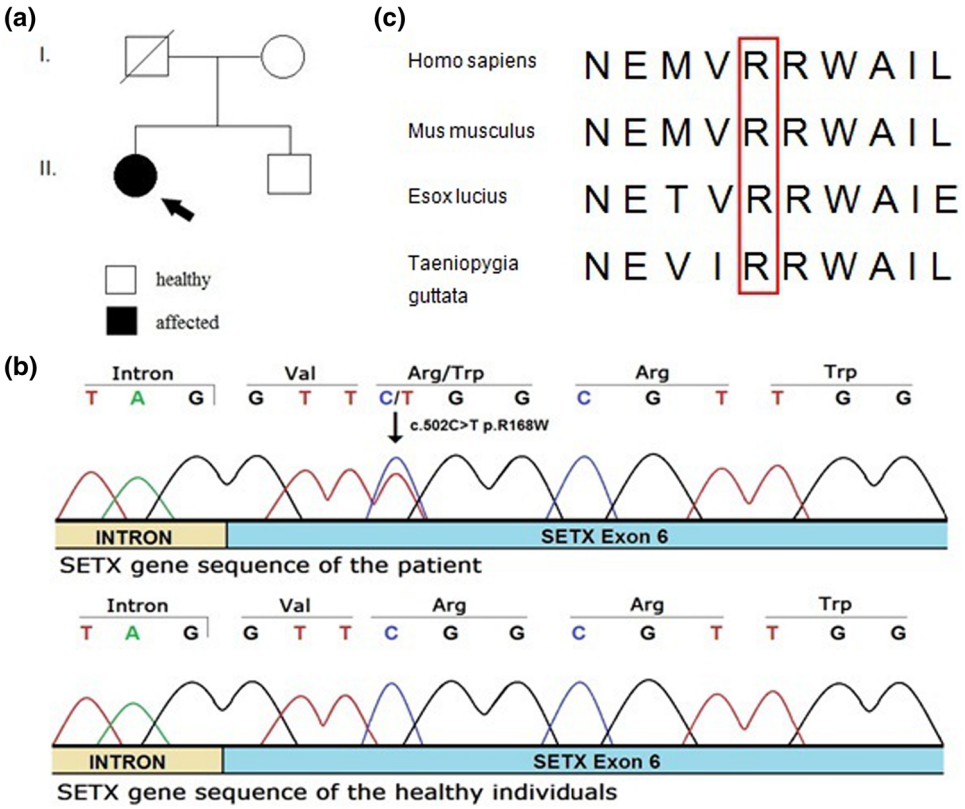

Fig. 1 **a** Family tree of the patient. **b** *Upper* DNA sequence of the patient, showing the heterozygous form of the exon 6 missense mutation c.502C>T p.Arg168Trp. *Lower* DNA sequence of the healthy individuals. **c** The identified amino acid change is located within a highly conserved region of the protein

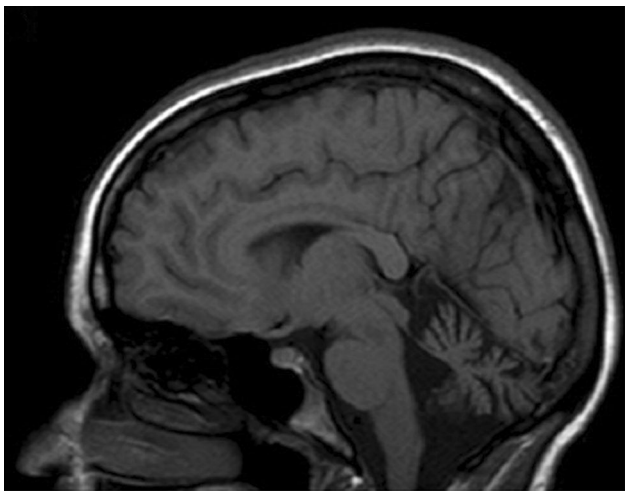

Fig. 2 Brain MRI of the patient, sagittal T1-weighted, indicating moderate cerebellar atrophy

exons 11–15. The presence of the c.502C>T variant in the ExAC database at a very low frequency (1/73,152 alleles in the European population) and the absence of homozygous healthy individuals are suggestive of its pathogenic effect (http://exac.broadinstitute.org/variant/9-135211899-G-A).

The identified arginine → tryptophan change caused by the missense mutation is located within a highly conserved region of the protein senataxin (Fig. 1c). We presume that this compound heterozygous state is responsible for the *SETX* insufficiency.

We were unable to perform the segregation analysis because the patient's father has already died and her closest relatives (mother and brother) did not give their consent to genetic testing.

Discussion

The majority of the AOA2 mutations reported earlier involved frameshift, missense, nonsense and splice-site mutations and deletions to various extents in the *SETX* gene. We report here a novel missense mutation and a large deletion in this gene, observed in a young woman with cerebellar ataxia, oculomotor apraxia, an elevated serum AFP level and cerebellar atrophy. This missense mutation, located outside the helicase domain of the protein senataxin, affected the *N*-terminal domain, while the extensive deletion gave rise to a truncating protein structure, including loss of the nuclear localization signal and in part the C-terminal helicase domain [2].

Table 1 presents the major clinical signs of 13 other AOA2 patients with exon 6 mutations [3–6] in comparison with the profile of our patient. Our patient manifested a

Table 1 Comparison of our patient with 13 other AOA2 patients with exon 6 mutations of the *SETX* gene [3–6]

	Data on 13 previously reported patients with exon 6 mutations	Our patient
Age at onset (years)	Mean: 12.7	25
Initial signs	Ataxia: 11/13 cases	Ataxia
	Dysarthria: 3/13 cases	
	Chorea: 2/13 cases	
	Head tremor: 1/13 cases	
Oculomotor apraxia	6/13 cases	Present
Other ocular signs	Strabism: 6/13 cases	Absent
	Nystagmus: 3/13 cases	
Tendon reflexes	Absent: 9/13 cases	Brisk
	Decreased: 4/13 cases	
Head tremor	4/13 cases	Absent
Deep sensory loss	9/13 cases	Absent
Cerebellar atrophy	Mild: 2/13 cases	Moderate
	Moderate: 6/13 cases	
	Severe: 3/13 cases	
	Not available: 2/13 cases	
AFP level (ng/ml)	32.9	21.9

later age at onset and brisk tendon reflexes relative to the other AOA2 cases. In our patient, the milder phenotype and the later onset of disease, compared to classic AOA2 cases, may be suggestive of a residual activity of *SETX* due to the hypomorph missense mutation.

The previous genotype to phenotype correlations coincides moderately with the clinical presentation of our patient. Despite the observation of brisk tendon reflexes in the lower limbs, pyramidal signs were more prevalent with missense mutations in the helicase domain. Dystonia was frequent, primarily with missense mutations in the helicase domain [2], and the lack of dystonia in our patient is therefore not surprising. Oculomotor apraxia was present in about half of the literature AOA2 patients and also in our patient. The elevated AFP levels and the cerebellar atrophy were significant diagnostic findings in almost all AOA2 cases, including our patient. The earlier studies reported that the phenotype was most severe when the mutation was missense and located outside the helicase domain, in contrast with the truncating mutations and the missense mutations affecting the helicase domain [2]. Our patient's symptoms are so far mild, possibly because of the compound heterozygous state and, respectively, the short time that has elapsed since the onset of the disease.

Acknowledgments This work was supported by KTIA Grant No. 13 NAP-A-II/17.

Compliance with ethical standards

Conflict of interest The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

Ethical approval This study was approved by the ethical Committee of the Faculty of Medicine, University of Szeged.

Informed consent Written informed consent was obtained from the patient for publication of this case report for educational purposes.

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II.

Journal of Human Genetics (2016), 1–5

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SHORT COMMUNICATION

Novel *AARS2* gene mutation producing leukodystrophy: a case report

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AARS2 **gene (NM_020745.3) mutations result in two different phenotypic diseases: infantile mitochondrial cardiomyopathy and late-onset leukoencephalopathy. The patient's first symptoms appeared at the age of 18 years with behavioral changes and psychiatric problems. Some years later, extrapyramidal symptoms, cognitive impairment, nystagmus, dysarthria and pyramidal symptoms also developed. The brain magnetic resonance imaging (MRI) indicated extensive white matter abnormalities. The diagnosis of** ***AARS2*** **gene mutations causing leukodystrophy was confirmed by genetic testing. Segregation analysis confirmed the compound heterozygous state of the patient. Histological examination of the biopsy did not prove specific pathological alterations. The clinical phenotype of our patient was compared with seven previously described patients suffering from leukoencephalopathy caused by** ***AARS2*** **mutations. We have documented a new, nonsense** ***AARS2*** **gene mutation (c.578T>G, p.Leu193*) and a known missense mutation (c.595C>T, p.Arg199Cys) associated with leukoencephalopathy in a male patient. Clinical features, imaging characteristics and genetic testing are presented, and histological data from an** ***AARS2*****-related leukodystrophy patient are described for the first time.**
Journal of Human Genetics advance online publication, 13 October 2016; doi:10.1038/jhg.2016.126

INTRODUCTION

Mitochondrial disorders are frequently caused by dysfunction of the oxidative phosphorylation system that results in energy deficit in the cells. This group of diseases is genetically heterogeneous as some of the mitochondrial proteins are encoded by the mitochondrial DNA, whereas the rest of them are translated from messenger RNAs transcribed from the nuclear genome. The mitochondrial DNA contains 22 mitochondrial transfer RNA (tRNA) and 2 mitochondrial ribosomal RNA encoding genes, in addition to 13 protein encoding genes that are subunits in the mitochondrial respiratory chain. Several proteins involved in mitochondrial translation, such as initiation, elongation and termination factors, tRNA-modifying enzymes and the mitochondrial aminoacyl-tRNA synthetases (ARS2) are encoded by nuclear genes.[1]

Two groups of aminoacyl-tRNA synthetases can be distinguished based on their mitochondrial or cytoplasmic localization. These enzymes are encoded by distinct nuclear genes, with the exception of GARS and KARS, because these genes encode the glycyl- and lysyl-tRNA syntethases in both positions. Aminoacyl-tRNA synthetases play a significant role in the initiation of translation by charging tRNAs with their appropriate amino acids. All of these proteins have a catalytic and an anticodon binding domain (see later aminoacylation domain), and some of them, including mitochondrial alanyl-tRNA synthetase, also have an editing domain that can deacylate mischarged amino acids.[2,3]

Mitochondrial alanyl-tRNA synthetase protein is encoded by the nuclear gene *AARS2*. Mutations of this gene result in two different phenotypic disorders that have been previously described. Certain mutations particularly affect the heart, producing a fatal, early-onset cardiomyopathy,[4–7] whereas others are associated with onset leukoencephalopathy and ovarian failure in females without cardiac involvement.[8,9] Euro *et al.*[7] stated that the cause of the distinct phenotypes is related to the activity of the aminoacylation, with cardiomyopathy resulting from a serious reduction in aminoacylation activity, and leukodystrophy resulting from only moderate reduction in activity.

To date, only seven patients have been identified with *AARS2*-associated leukoencephalopathy and only one of them is male.

Here we report a young Caucasian male patient with a leukodystrophy phenotype and a novel *AARS2* gene mutation.

CASE PRESENTATION

Patient history and clinical data

The patient is a 29-year-old male with an uneventful perinatal period. His grandparents, parents and his younger brother did not report any neurological or psychiatric problems. The parents noted that the patient's behavioral changes began at the age of 18 years. At that time he exhibited an alteration of personality, mania and paranoid delusions. Therefore, he was admitted to a psychiatric unit, where he underwent antipsychotic treatment. Some years later, extrapyramidal symptoms appeared and they were attributed to the side effects of the antipsychotic drugs. Otherwise, his somatic and

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Received 7 July 2016; revised 12 September 2016; accepted 12 September 2016

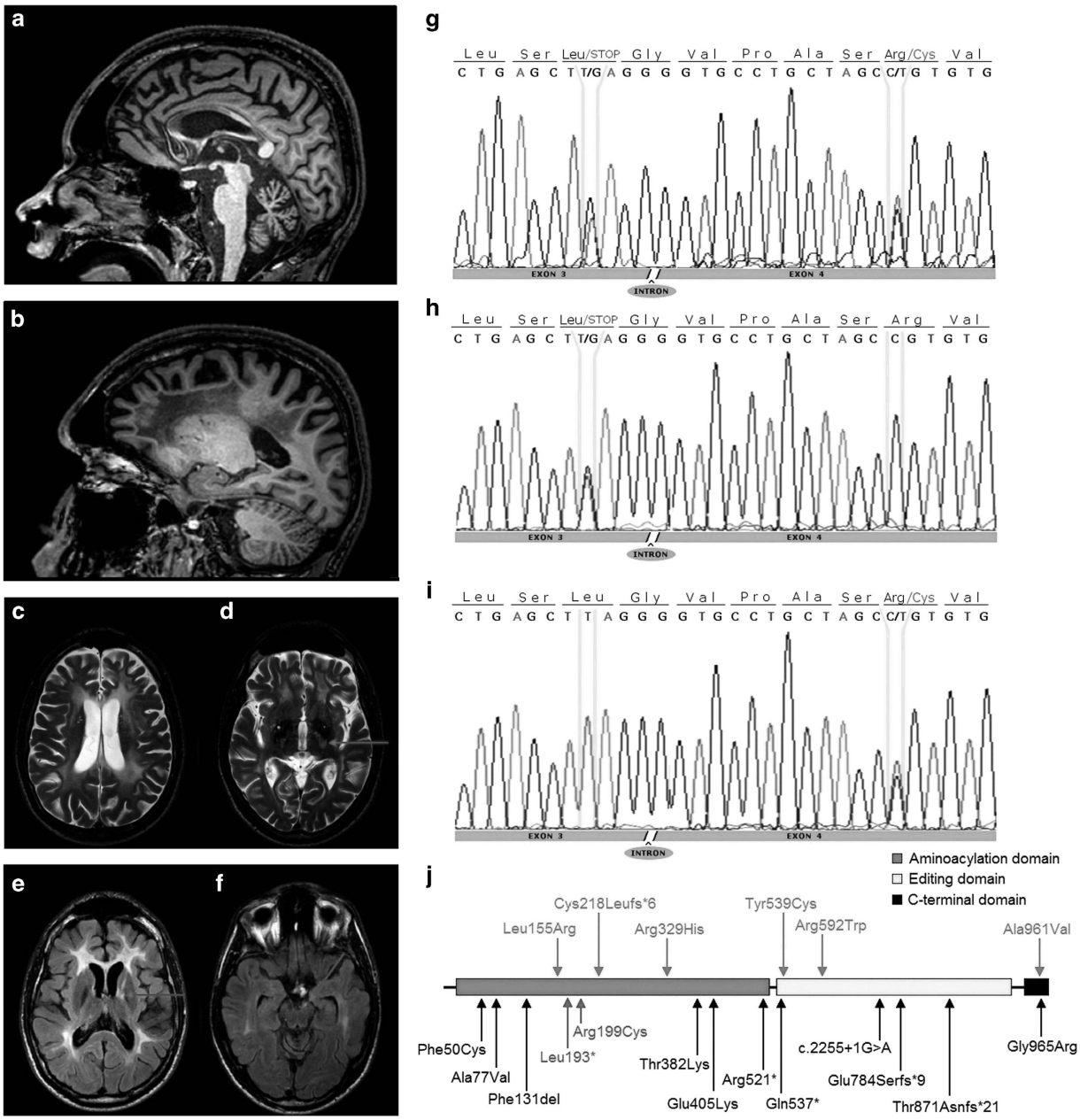

Figure 1 (**a**–**f**) Brain magnetic resonance imaging (MRI) of the patient, (**g**–**i**) *AARS* gene sequencing of the patient and his parents and (**j**) structure of the *AARS2* gene. (**a**, **b**) T1-weighted, sagittal scans show corpus callosum involvement mainly in the frontal part and extended white matter abnormalities in the frontoparietal region with relative sparing of the central area. (**c**, **d**) T2-weighted, axial scans demonstrate pronounced leukoencephalopathy. The arrow proves the lesion of pyramidal tract in level of internal capsule. (**e**, **f**) Fluid-attenuated inversion recovery (FLAIR)-weighted, axial scans: the arrows show the frontopontine tract involvement in the level of internal capsule and the cerebral peduncle. *AARS2* gene sequence of the patient (**g**) showing both mutations (c.578T>G and c.595C>T). DNA sequence of the patient's father (**h**) and mother (**i**) demonstrating only one mutation, confirming the compound heterozygosity of the subject. Genomic structure of *AARS2* (**j**) with exons coding for the aminoacylation (green), editing (yellow) and C-terminal (black) domains. The blue arrows (upper) indicate the position of mutations causing infantile mitochondrial hypertrophic cardiomyopathy. The lower arrows indicate the position of mutations causing leukoencephalopathy. The mutations of our patient indicated red color, whereas the other mutations resulting in leukodystrophy signed black color. The nonsense mutation (p.Leu193*) was reported here for the first time, whereas the missense mutation (p.Arg199Cys) has been described previously.[5,6,8] A full color version of this figure is available at the *Journal of Human Genetics* journal online.

intellectual development was normal until age 24 years, when signs of cognitive impairment became obvious. Before this deterioration, the patient was an excellent student in secondary school and also in college. There was a rapid and progressive cognitive decline, and during the following 2 years he evolved acalculia, orientation problems and dysgraphia. Besides these abnormalities, the neurological examination revealed horizontal and vertical nystagmus, dysarthria, dysphagia and extrapyramidal symptoms, including rigor, hypo- and bradykinesia, pyramidal symptoms such as Babinski sign and brisk tendon reflexes and frontal release signs, including glabellar, palmomental and sucking reflex and palmar grasp. Ophthalmoscopy and cardiological investigation did not reveal any pathological signs.

The patient is still alive and is currently bedridden because of the severe neurological deficits.

MRI abnormalities

The brain magnetic resonance imaging (MRI) represented extensive white matter abnormalities, predominantly present in the frontal and parietal lobe, with a relative sparing of the central region. The corpus callosum was affected as well, especially the frontal part. Pyramidal tract involvement is represented at the level of the internal capsule. In addition, the frontopontine fibers are specifically affected within the internal capsule and in the brainstem (Figures 1a–f).

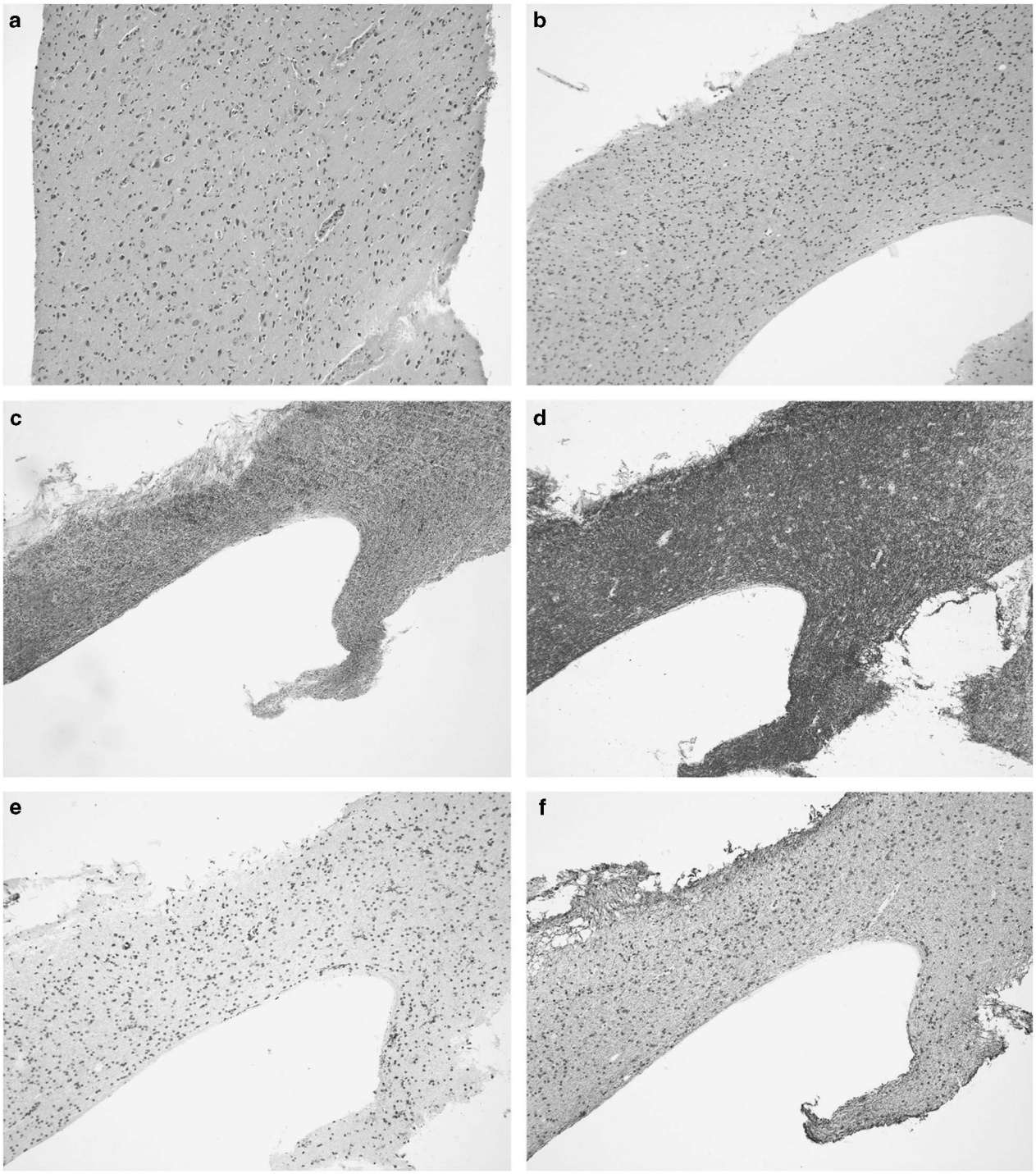

Figure 2 Representative histopathological images of the biopsy sample. (**a**) Hematoxylin and eosin (HE) staining of the cortex reveals preserved neurons. HE (**b**), Klüver–Barrera (**c**), immunostaining for myelin basic protein (**d**), HLA-DR (microglia marker) (**e**) and SMI-31 (neurofilament) (**f**) of the white matter does not show disease-specific alterations, inflammation or demyelination. A full color version of this figure is available at the *Journal of Human Genetics* journal online.

Table 1 Comparison of the clinical phenotypes of our patient with the earlier described seven patients suffering from *AARS2* mutation causing leukoencephalopathy

	P1	*P2*	*P3*	*P4*	*P5*	*P6*	*P7*	*Our patient*
Age at first neurological symptom(s) (years)	3	7	33	24	40	22	30	18
First neurological symptom(s)	Impaired balance	Learning difficulties	Depression, cognitive decline	Tremor of the hands, cognitive decline, behavioral changes	Depression, cognitive deterioration	Gait problems	Cognitive decline, behavioral changes	Paranoid delusions, personality changes
Cerebellar symptoms	+	+	+	+	−	+	+	+
Cognitive deterioration	+	+	+	+	+	−	+	+
Psychiatric symptoms	+	−	+	+	+	+	+	+
Pyramidal signs	+	+	−	+	−	+	+	+
Dystonia	−	+	−	+	−	−	−	−
Epilepsy	−	−	+	−	−	−	−	−
Ovarian failure	+	Male	+	+	+	+	+	Male

The symbol '+' indicates present and the symbol '−' indicates not present.
P1–7 patients have been described earlier with *AARS2* mutation causing leukodystrophy.

Laboratory findings

Numerous examinations were performed to determine the precise diagnosis of this early-adulthood-onset leukodystrophy. The laboratory findings, including a complete blood count, creatine kinase and serum lactate, were normal. The parameters of the cerebrospinal fluid, including leukocytes, protein content and IgG index, were within the normal range, and isoelectric focusing did not display any abnormalities. PCR tests of the herpes simplex virus types 1 and 2 from the cerebrospinal fluid and the HIV-1 test from blood were negative. The serum levels of very long chain fatty acids and phytanic acid were not elevated. The arylsulfatase-A and galactocerebrosidase activity from leukocytes showed intact function of these enzymes. The blood mass spectrometry for amino acids, acylcarnitine and succinylacetone did not point to metabolic deviations. Filipin staining in cultured fibroblasts did not confirm the diagnosis of Niemann–Pick disease type C.

Genetic tests

Genetic testing for the most common vanishing white matter disease causing gene *EIF2B5* was also negative. Earlier genetic examinations did not verify CADASIL (Cerebral Autosomal-Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy) disease. Based on the phenotype and the characteristic MRI features, targeted gene sequencing of *AARS2* (NM_020745.3) was performed. The sequencing identified three variations: c.578T>G, c.595C>T and c.2188G>A (Figure 1g). The c.578T>G single-nucleotide polymorphism can be considered a pathogenic variation as it causes a nonsense mutation (p.Leu193*) in exon 3 and is likely to result in messenger RNA degradation by nonsense-mediated decay. This mutation was not described earlier and it could not be found in the ExAC database (The Exac Database; http://exac.broadinstitute.org/gene/ENSG00000124608). The c.595C>T variation causes a missense mutation (p.Arg199Cys) in exon 4 of the gene and its presence was also detected in four unrelated patients with similar clinical presentation.[8] The frequency of this allele is 14/72 800 in the European population (The Exac Database; http://exac.broadinstitute.org/variant/6-44278885-G-A). The c.2188G>A single-nucleotide polymorphism also results in a missense mutation (p.Val730Met), but it is considered a polymorphism based on its high allele frequency in control subjects (~4% in the European population; The Exac Database; http://exac.broadinstitute.org/variant/6-44270870-C-T). Segregation analysis indicated that the pathogenic mutations are not in the same allele of the *AARS2* gene, as the patient's father carries only the c.578T>G nonsense mutation, whereas his mother bears only the c.595C>T mutation. Consequently, the patient has a compound heterozygous mutation resulting in a late-onset leukodystrophy (Figures 1h and i). Figure 1j shows the aminoacylation, the editing and the C-terminal domains of the *AARS2* gene with our patient's and the earlier described mutations.

Histology

Before the identification of the genetic abnormality, a biopsy sampling from the frontal lobe, including cortex and white matter, was performed by another department. There was a lack of necrotic damage, inflammatory infiltration, neuronal changes or vascular lesions in the hematoxylin–eosin staining. In the Klüver–Barrera myelin staining, together with the immunostaining for myelin basic protein and neurofilament, we did not observe selective demyelination or axonal changes. Furthermore, there was a lack of amyloid precursor protein immunoreactive axonal bulbs. Immunostaining for CD3 (T cells), CD8 (cytotoxic T cells) and CD20 (B cells) was completely negative. There was an absence of macrophage activity (CD68) and only very minor microglial reaction was noted (HLA-DR). Screening for pathological protein inclusion using immunostaining for ubiquitin and p62 did not reveal unequivocal pathological deposits (Figure 2). In summary, this biopsy did not indicate pathological alterations, in particular not selective demyelination or axonal/myelin pathology.

Ethics approval and consent to participate

This study was approved by the ethical committee of the Faculty of Medicine, University of Szeged. Written informed consent was obtained from the patient for publication of this case report for educational purposes.

DISCUSSION

In this paper we describe a patient with an early-adulthood-onset leukodystrophy caused by a compound heterozygous mutation of the *AARS2* gene. One of these pathogenic mutations (c.578T>G) has not been previously published, whereas the other (c.595C>T) has been detected in four unrelated patients with leukodystrophy.[8] To date, *AARS2* mutations have been reported in 11 subjects who have infantile mitochondrial hypertrophic cardiomyopathy with early fatal outcomes and 7 patients with late-onset leukodystrophy.[4–9] Our patient's phenotype is consistent with the predictions described by Euro *et al.*[7]

in 2015 that one nonsense mutation together with the milder p.Arg199Cys mutation cause the phenotype of leukodystrophy. Similar to the previous observation, our patient did not have any cardiac symptoms and his symptoms began after childhood and progressed rapidly. Behavioral changes were observed first and then neurological symptoms appeared, including progressive cognitive decline, frontal lobe dysfunction, cerebellar and extrapyramidal signs. The brain MRI abnormalities supported these symptoms, with extensive white matter lesions of frontal and parietal predominance, whereas corpus callosum, brainstem, pyramidal tract and frontopontine fiber involvement were observed. The clinical and radiological findings of our patient are similar to the subjects whose *AARS2* gene mutation caused leukodystrophy (Table 1). As the biopsy sample included a region that was not severely affected, histopathological analysis did not reveal any disease-specific alteration.

In summary, ovarian failure and late-onset leukoencephalopathy raise the suspicion of the diagnosis of *AARS2*-associated leukodystrophy and vanishing white matter disease. The characteristic MRI abnormalities, the described neurological symptoms and the progressive course of the disease could help in differentiation. This novel form of leukodystrophy is extremely rare, with our patient being the eighth patient in the literature and only the second male patient. In this report we have identified a new, not previously published disease-causing mutation and the very first histological data of this disease.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We thank Professor Marjo S van der Knaap, VU University Medical Center, Amsterdam, The Netherlands, for her help. This work was supported by Hungarian Brain Research Program KTIA Grant No. 13 NAP-A-II/17, KTIA Grant No. 13 NAP-A-II/18 and NKFI Grant K-112294.

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III.

Neurol Sci
DOI 10.1007/s10072-016-2776-6

BRIEF COMMUNICATION

Different phenotypes in identical twins with cerebrotendinous xanthomatosis: case series

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Received: 5 September 2016 / Accepted: 18 November 2016

Abstract Cerebrotendinous xanthomatosis (CTX) is a rare, genetically determined error of metabolism. The characteristic clinical symptoms are diarrhea, juvenile cataracts, tendon xanthomas and neuropsychiatric alterations. The aim of this study is to present a pair of identical adult twins with considerable differences in the severity of phenotype. With regards to neuropsychiatric symptoms, the predominant features were severe Parkinsonism and moderate cognitive dysfunctions in the more-affected individual, whereas these alterations in the less-affected patient were only very mild and mild, respectively. The characteristic increase in the concentrations of serum cholestanol and the lesion volumes in dentate nuclei in the brain assessed with magnetic resonance imaging were quite similar in both cases. The lifestyle conditions, including eating habits of the twin pair, were quite similar as well; therefore, currently unknown genetic modifiers or certain epigenetic factors may be responsible for the differences in severity of phenotype. This case series serves as the first description of an identical twin pair with CTX presenting heterogeneous clinical features.

Keywords Cerebrotendinous Xanthomatosis · Parkinsonism · Cognitive dysfunction · Identical twins · Heterogeneous phenotype

Introduction

Cerebrotendinous xanthomatosis (CTX) is an autosomal recessively inherited condition belonging to the group of inborn errors of metabolism [1]. It affects approximately 1–2 out of 100,000 individuals. The pathogenic mutations are located in the *CYP27A1* gene mapped to 2q35 [2, 3]. The gene product, sterol 27-hydroxylase, which is expressed in the central nervous system, liver, lungs, duodenum and endothelial cells, takes part in the appropriate production of bile acids from cholesterol [4]. Sterol 27-hydroxylase deficiency results in the accumulation of 7α-hydroxy-4-cholesten-3-one and its metabolites, including cholestanol, and also results in the insufficient production of chenodeoxycholic acid [1]. The main characteristic signs and symptoms of CTX are diarrhea, juvenile cataracts, tendon xanthomas and neuropsychiatric alterations, including cognitive and psychiatric disturbances, pyramidal and/or cerebellar signs, seizures and Parkinsonism [3]. Although there is a marked heterogeneity of signs and symptoms in CTX patients, even in intrafamilial cases [2], no phenotypic variability has yet been reported in twins or triplets, [5]. The aim of the current study is to present the considerably different phenotypes of a pair of identical adult twins diagnosed with CTX.

Electronic supplementary material The online version of this article (doi:10.1007/s10072-016-2776-6) contains supplementary material, which is available to authorized users.

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Published online: 25 November 2016

Case reports

One member of a 40-year-old female twin pair was first admitted to our neurology department in 2016 with the aim of a diagnostic work-up on her movement disorder. She had already had cataracts and glaucoma in childhood, but her movement and speech only began to worsen progressively from 2013. In addition, she complained of an episode of pronounced diarrhea and gastrointestinal discomfort in 2015, and her parents mentioned memory problems, anxiety and impatience as well. On neurological examination, she presented signs of a movement disorder with dominating Parkinsonism (moderate symmetric hypo- and bradykinesia, rigor, mainly right-sided occasional limb rest tremor, severe postural instability, freezing of gait, antecollis, hypomimia, severe dysarthria and mild seborrhea; Movement Disorder Society Unified Parkinson's Disease Rating Scale (MDS-UPDRS) part III: 75 points in OFF state; Suppl. video 1) showing some levodopa response (MDS-UPDRS part III: 56 points in ON state, i.e., 25.3% improvement; Suppl. video 2). Furthermore, the patient had mild cerebellar ataxia and pyramidal signs (brisk patellar reflex and ankle clonus on the right side with bilateral Babinski sign) as well. The unaided stance and gait could not be implemented. The neuropsychological assessment demonstrated moderate cognitive dysfunctions in light of 65/100 points in Addenbrooke's Cognitive Examination (ACE) and 24/30 points in the Mini-Mental State Examination (MMSE). The skull MRI revealed T2 and FLAIR abnormal signals in the dentate nuclei and some supratentorial white matter alterations (Fig. 1a–d). The clinical picture raised the possibility of CTX which was supported by elevated serum cholestanol levels (31 μM; normal range 2–12.6 μM). The genetic testing for a disease-causing mutation in the *CYP27A1* gene revealed a known pathogenic homozygous frameshift mutation in exon 4 (c.819delT, p.D273EfsTer13).

With regards to family history, the other member of the twin pair also had juvenile cataracts and glaucoma and was suspected to have some kind of immunological disorder. Furthermore, her parents also reported less-expressed memory problems, anxiety and impatience. Otherwise, the family history of the assessed patient was irrelevant. Despite the absence of pronounced neurological features, this second patient was also screened for possible signs of CTX. The examination revealed signs of slight Parkinsonism (mainly left-sided slight hypo- and bradykinesia and rigor on provocation in the left upper limb, and

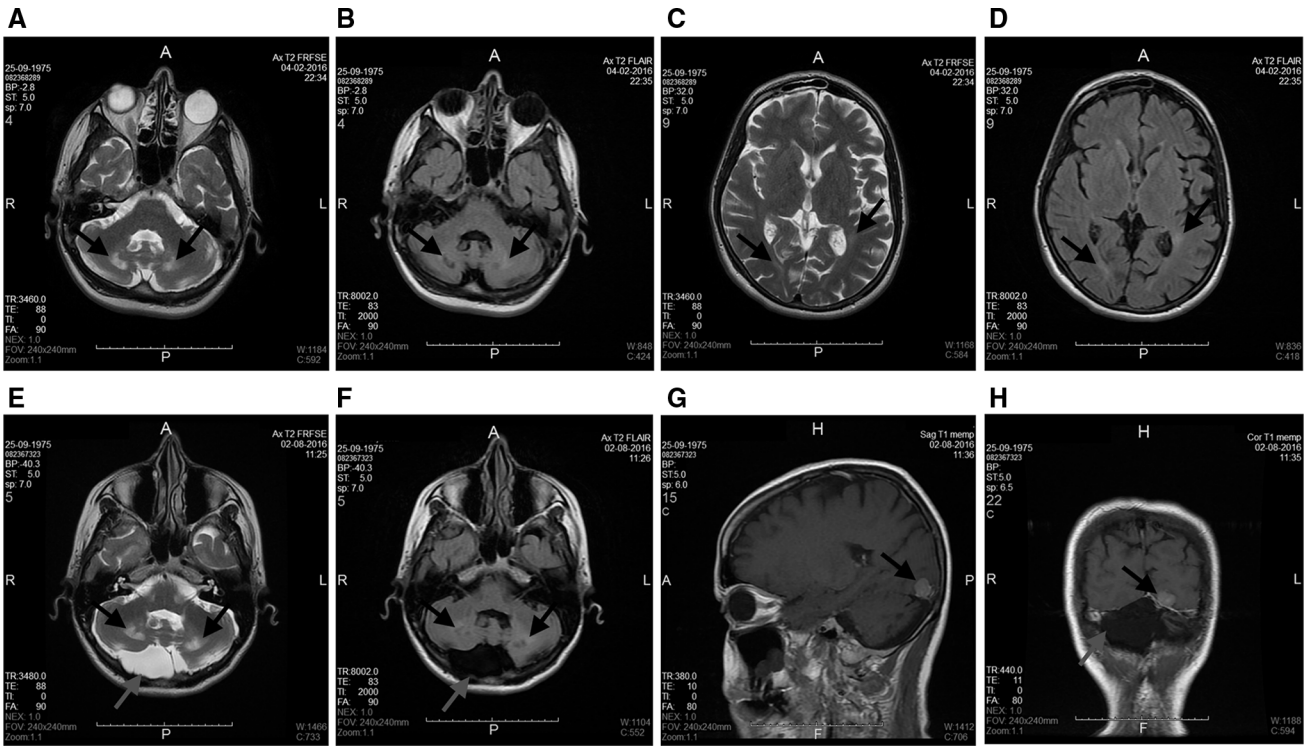

Fig. 1 The main characteristic magnetic resonance imaging (1.5 T) features of our patients with cerebrotendinous xanthomatosis. The lesion of the dentate nuclei is prominent in the more-affected member (*black arrows*; T2-weighted images: **a**; FLAIR images: **b**) and the less-affected member (*black arrows*; T2-weighted images: **e**; FLAIR images: **f**) of the identical twin pair. Furthermore, the more-affected member demonstrated supratentorial white matter alterations (*black arrows*; T2-weighted images: **c**; FLAIR images: **d**). In the less-affected member, the MRI revealed asymptomatic mega cisterna magna (*grey arrows*; axial T2-weighted images: **e**; axial FLAIR images: **f**; coronal T1-weighted images: **h**) and a small contrast-enhancing tentorial meningioma (*black arrows*; sagittal T1-weighted images: **g**; coronal T1-weighted images: **h**)

moderate postural instability; MDS-UPDRS part III: 8 points in OFF state; Suppl. video 3) and mild sensory ataxia with a slightly broad-based gait. With regards to cognitive function, the neuropsychological assessment demonstrated mild alterations in light of 72/100 points in ACE and 27/30 points in the MMSE. The abnormal signals in the dentate nuclei were present as well (Fig. 1e, f). Furthermore, the MRI revealed asymptomatic mega cisterna magna (Fig. 1e, f, h) and a small tentorial meningioma (Fig. 1g, h). Despite the less-expressed clinical alterations when compared to her twin sister, similarly elevated serum cholestanol levels were detected (36.8 μM). The genetic testing identified the same disease-causing mutation in the *CYP27A1* gene.

The considerably different phenotypes raised the question of whether the twin pair is identical or not. The following 15 short tandem repeat markers were analyzed for that purpose: D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TC11, D13S317, D16S539, D2S1338, D19S433, VWA, TPOX, D18S51, D5S818 and FGA. The DNA profile of the two subjects was completely identical, so they are confirmed to be identical twins. The parents were heterozygous for the assessed mutation and they did not show any sign or symptom of CTX.

Discussion

Although the role of *CYP27A1* gene mutations in the pathogenesis of CTX is well-established, no genotype–phenotype correlation can be determined. When compared with previously reported CTX patients, the clinical features of our identical twin pair were slightly different from typical cases (including those with the same pathogenic mutation [6]), lacking tendon xanthomas and seizures, and presenting a predominant Parkinsonian syndrome, especially in the more severely affected patient. However, the Parkinsonian features were similar to that of a previously published case [7]. Nevertheless, the major aim of this case series is not only the simple demonstration of signs and symptoms in this identical twin pair, but to draw attention to the considerable differences in the severity of their clinical features and the identification of possible underlying factors. Biochemical testing revealed similar alterations in cholestanol concentrations, but it is known that the levels of this metabolite have no correlation with clinical phenomena [8]. Although the role of environmental factors has been suggested to be possibly responsible for the clinical differences [2], the fact that our twin pair has been continuously living together with their parents and their eating habits are similar as well, makes this theory questionable. However, currently unknown genetic modifiers of certain epigenetic factors may play a role in clinical heterogeneity. The clarification needs further studies.

In conclusion, this case series study serves as the first report of identical twins diagnosed with CTX demonstrating remarkably different phenotypes.

Acknowledgements This study was funded by Hungarian Brain Research Program—Grant No. KTIA_NAP_13-A_II/18. Dénes Zádori was supported by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences. The linguistic corrections were made by a native English reader, Jennifer Tusz from Canada.

Compliance with ethical standards

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

Ethical approval Written informed consent was obtained from the patients for video recording and the publication of this study (institutional research committee registration numbers are 150/2014. and 44/2016., respectively). All procedures applied during the assessment of patients were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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IV.

Cerebellum
DOI 10.1007/s12311-017-0870-9

SHORT REPORT

Neurocognitive Characterization of an SCA28 Family Caused by a Novel *AFG3L2* Gene Mutation

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Introduction

Autosomal, dominantly inherited spinocerebellar ataxias (SCAs) are a continuously expanding, clinically and genetically heterogeneous group of neurological disorders. Prevalence of the autosomal dominant cerebellar ataxias (ADCAs) is estimated to be approximately 1–5:100,000 population [1, 2]. The main neurological symptoms are gait and limb ataxia and dysarthria, accompanied by additional neurological signs, which are variable and often overlapping within the subtypes of the group. The characteristic cognitive abnormalities are executive dysfunction and visuospatial disability in the most common SCAs (SCA1, 2, and 3) [3, 4]. The genetic diversity of SCAs comprises trinucleotide repeat expansions (SCA1, 2, 3, 6, 7, 8, 12, 17, and dentatorubral-pallidoluysian atrophy), pentanucleotide repeat expansions (SCA10 and 31), hexanucleotide repeat expansions (SCA36), and conventional mutations (SCA5, 11, 13, 14, 15/16, 18, 19/22, 21, 23, 26, 27, 28, 29, 34, 35, 38, and 40), whereas the responsible gene has not yet been identified in some forms of SCAs (SCA4, 20, 25, 30, 32, and 37) [5].

SCA28 was characterized in 2006 by Cagnoli et al. as a juvenile-onset, slowly progressive gait and limb ataxia with eye movement abnormalities [6]. The causative gene, *AFG3L2* (ATPase family gene 3-like 2), containing 17 exons, was discovered in 2010 by Di Bella et al. and encodes the mitochondrial metalloprotease AFG3L2 [7]. The AFG3L2 protein is a zinc-dependent metalloprotease which contains 797 amino acids forming four different domains and plays an important role in the quality control of mitochondrial proteins [7–9]. Most of the identified *AFG3L2* mutations are missense and located in exons 15 or 16 of the gene. All SCA28 cases were identified in the Caucasian population, except for one patient from Africa reported in the literature [6–8, 10–18]. There is a lack of thorough cognitive characterization of SCA28 patients in the literature. Most of the studies revealed normal cognitive functions in SCA28, while mild cognitive impairment or decreased intelligence quotient is uncommon [19].

Here, we report the detailed neurological and cognitive assessment of the first Hungarian family identified with a novel *AFG3L2* mutation producing SCA28. The purpose of the study was to describe a new pathogenic mutation and to perform a clinical characterization of the family with SCA28, especially the cognitive aspects. With regard to the extreme rarity of the disease, the expectation of finding a larger sample is certainly not great, so these data are called as a pilot study of a small group of subjects with SCA28.

Patients and Methods

Clinical Assessment

Five affected patients from a Hungarian family were assessed with the suspicion of hereditary ataxia. The neurological investigation was performed by a movement disorder specialist.

Electronic supplementary material The online version of this article (doi:10.1007/s12311-017-0870-9) contains supplementary material, which is available to authorized users.

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Published online: 28 June 2017

The family tree was also delineated (Fig. 1). Routine laboratory examination was carried out on all the affected subjects, while brain MRI was performed on four out of five patients.

Genetic Testing

Targeted Gene Analysis Followed by Clinical Exome Sequencing

After obtaining written, informed consent, genomic DNA was extracted from peripheral blood leukocytes by standard protocol. First, the most common SCAs (SCA1, 2, 3, 6, and 7) were tested in four out of five patients by CAG repeat expansion analysis.

For clinical exome sequencing, a total of 60 ng of genomic DNA was used for library preparation and sequenced with TruSight One clinical exome kit (Illumina) on Illumina MiSeq platform. The clinical exome kit covers the coding region of 4813 clinically relevant, disease-associated genes.

The 150-bp paired reads were aligned to the GRCh37.75 human reference genome by Burrows Wheel Aligner (BWA v0.7.9a) software. The variants were called by Genome Analysis Toolkit HaplotypeCaller (GATK v3.5) best practice and annotated by SnpEff and VariantStudio softwares. Variants were filtered based on severity and frequency against public variant databases including dbSNP, ClinVar, ExAC, EVS, and an in-house clinical exome database of 140 unrelated Hungarian patients.

Cognitive Characterization

Cognitive assessment was performed on all the affected patients. The following major neuropsychological functions were examined: phonological immediate memory, visuospatial immediate memory, working memory, executive functions, semantic memory, visual attention, and speed of processing.

First, to obtain a brief global cognitive assessment, Addenbrooke's cognitive examination (ACE) incorporating the Mini-Mental State Examination (MMSE) was performed [20]. Phonological immediate memory was measured with the Digit Span Task (DST) [21]. Visuospatial immediate memory was assessed with the Corsi Block-Tapping Test (CBTT) and the Brief Visuospatial Memory Test-Revised (BVMT-R) [22, 23]. Working memory was measured with the Backward Digit Span Task (BDST) and the Listening Span Task (LST) [24–26]. Letter, verb, episodic, and semantic fluency tests and the Wisconsin Card Sorting Test (WCST) were performed as well to assess executive functions [27–29]. Everyday memory functions, including semantic memory, were measured with subtests of the Rivermead Behavioural Memory Test (RBMT) [30]. Visual attention and task switching functions were investigated with the Trail Making Test (TMT) [31]. The Hamilton Rating Scale for Depression (HRSD) was completed as well by two out of five patients.

Results

Clinical Assessment

Five affected patients from a Hungarian family were assessed with the suspicion of hereditary ataxia. First, the family tree was delineated, which suggested an autosomal dominant inheritance pattern (Fig. 1). The first symptom of the proband (patient 1, IV-5) was observed at the age of 15 years as clumsiness of the limbs which exclusively occurred after

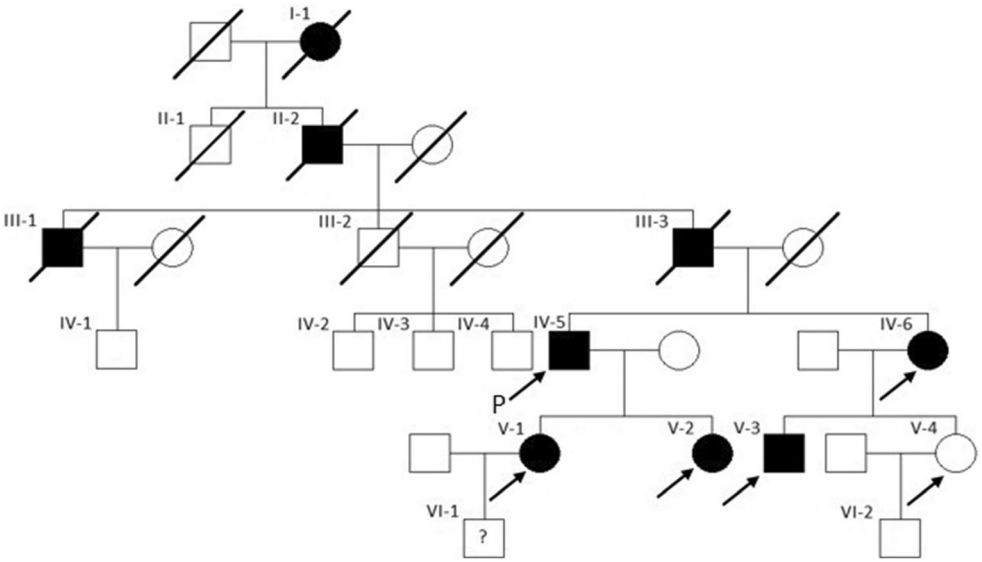

Fig. 1 Pedigree of the assessed family. The generations are indicated with *Roman numbers* while individuals in each generation with *Arabic numbers*. The deceased members are *crossed*. The patients demonstrating symptoms are in *black*. The proband is indicated with *P*, whereas the individuals seeking genetic testing with *arrows*

pronounced physical stress. In the following years, he developed uncoordinated gait, speech disturbance, and mild double vision. The first complaints of the proband's sister (patient 2, IV-6) occurred as writing difficulty, gait difficulty, and problems with speech when she was 28 years old. In the next generation of the family, the first symptoms of all the affected subjects were provoked by physical activity in high school scheduled physical education lessons. Actually, patients 3 (V-3) and 4 (V-1) have complaints without physical stress as well, while patient 5 (V-2) does not. Neurological examination of the patients revealed cerebellar symptoms of varying severity with dysarthria and eye movement abnormalities. Table 1 demonstrates the detailed neurological characterization of patients.

Routine laboratory parameters were in the normal range, except slightly elevated serum total cholesterol levels in patients 1 and 2, and minimally increased serum creatine kinase levels in patients 2 and 3. The brain MRI showed mild to moderate cerebellar atrophy mainly in the vermis in patients 1–4 (Fig. 2). Brainstem and supratentorial structures did not demonstrate pathological alterations.

Genetic Testing

According to autosomal dominant inheritance, the most common SCAs were investigated first in patients 1–4 by CAG repeat expansion analysis. The CAG repeat numbers of SCA1, 2, 3, 6, and 7 were in the normal range, so in the next step, clinical exome sequencing was performed for patient 1 (IV-5; Fig. 1). Searching for possible causative variants in the 48 SCA-associated genes, we identified 129 variants. Out of 41 non-synonymous variants, 12 were rare (based on available public databases ExAC and dbSNP) from which only three have autosomal dominant inheritance. We excluded *ITPR1* NM_001168272.1:c.1435G>A (rs41289628) and *CACNA1A* NM_023035.2:c.6976 6993delCAGCAGCAGCAGCA

Table 1 Neurological characterization of the patients

	Patient 1 (IV-5)	Patient 2 (IV-4)	Patient 3 (V-3)	Patient 4 (V-1)	Patient 5 (V-2)
Age at onset (years)	15	28	14–18	14–18	14–18
Age at examination (years)	60	59	31	31	27
Gender	Male	Female	Male	Female	Female
Gait ataxia	++	++	+	+	+
Upper limb ataxia	+	+	+	+	+
Lower limb ataxia	+++	++	++	+	+
Dysarthria	++	++	+	+	+
Broken up smooth pursuit	P	P	P	P	P
Gaze-evoked nystagmus on horizontal testing	P	P	P	P	P
Upbeat nystagmus on vertical testing	P	P	–	–	–
Ophthalmoparesis	–	–	–	–	–
Ptosis	–	–	–	–	–
Slowing of saccades	–	–	–	–	–
Impaired visual acuity	P	P	–	–	–
Double vision	++	–	+	–	–
Dysphagia	++	+	–	+	–
Paresis	–	–	–	–	–
Deep tendon reflexes	Brisk	Normal	Normal	Brisk	Brisk
Extensor plantar reflex	–	–	–	–	–
Hypotonia	+	+	+	++	+
Muscle atrophy	–	–	–	–	–
Chorea, myoclonus, dystonia	–	–	–	–	–
Rigidity	–	–	–	–	–
Resting tremor	–	–	–	–	–
Impaired vibration sense	–	–	–	–	–
Incontinence	–	–	–	–	–
SARA score	14/40	10/40	7.5/40	7.5/40	4.5/40

SARA scale for the assessment and rating of ataxia, + mild, ++ moderate, +++ severe, *P* present, − not present

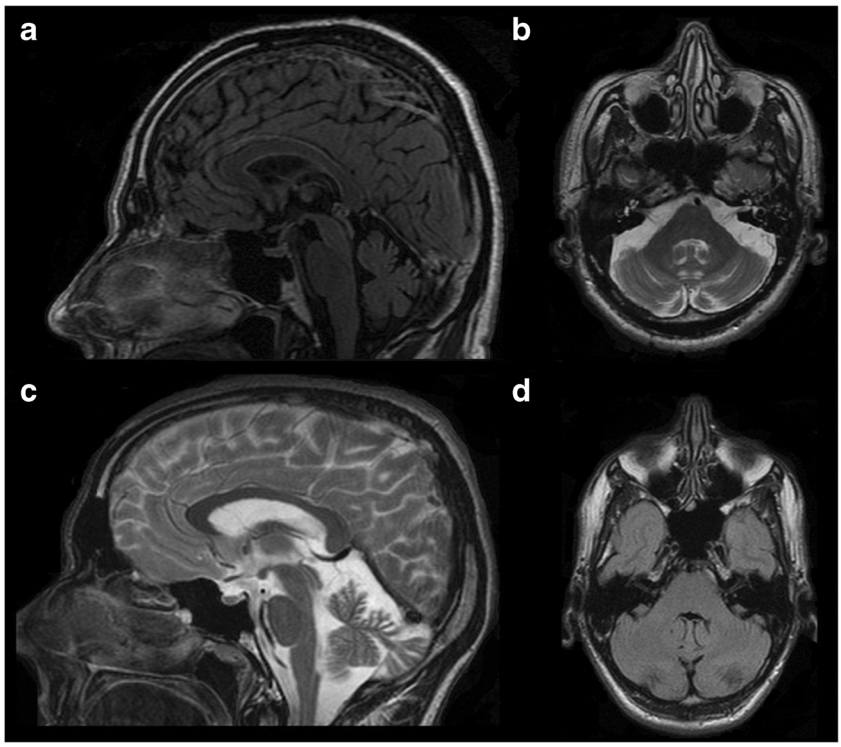

Fig. 2 Brain MRIs of patients 1 and 3. Sagittal, FLAIR-weighted (**a**) and axial, T2-weighted (**b**) images of patient 1 demonstrate mild cerebellar atrophy, mainly in the vermis. Sagittal, T2-weighted (**c**) and axial FLAIR-weighted (**d**) pictures of patient 3 indicate moderate cerebellar atrophy

GCAGinsCAGCAGCAGCAGCAG as possible causative mutations as both were found among unaffected Hungarian controls (2/148) as well.

Thus, we identified a heterozygous missense variant c.2011G>C p.Gly671Arg in *AFG3L2* gene (Fig. 3a). This novel mutation was not found either in the 148 unrelated Hungarian controls or in dbSNP and ExAC databases and was not previously described in studies focused on *AFG3L2* mutations in SCA28 [7, 8, 10–18]. The presence of this mutation was confirmed by targeted Sanger sequencing in patient 1 (IV-5) and in four affected, living relatives (IV-6, V-1, V-2, and V-3; Fig. 1 and 3) but was not found in a healthy sister (V-4) of patient 3 (Fig. 3a). The variant is located in exon 16 of the gene (Fig. 3c). In this position, two other pathogenic nucleotide changes were detected earlier: c.2011G>A p.Gly671Arg by Cagnoli et al. and c.2011G>T p.Gly671Trp by Gorman et al. [8, 11]. The identified glycine to arginine amino acid change caused by the missense mutation is located within a highly conserved Peptidase M41 region of the protein AFG3L2 (Fig. 3b).

Cognitive Characterization

Supplementary Table 1 shows the results of the cognitive examination, performed on all the affected patients. Summarily, the patients demonstrated normal cognitive performance when global assessment was carried out (90–96/100 point in ACE). However, they performed slightly lower in certain aspects relative to their age and level of education. The affected cognitive functions included complex working memory (LST and BDST), visuospatial memory (CBTT), semantic memory (RBMT immediate recall of the story), and executive functions (based on their performance in letter fluency). In the eldest patient (patient 1), the alteration in executive functions was also supported by the fact that he made several mistakes in TMT B which he completed in a remarkably longer period of time, and he made mistakes during the clock-drawing test as well. Patient 1 performed at a lower level in WCST for several aspects of the task. Patient 2 also demonstrated a mild deficit in executive functions based on letter fluency tests and WCST. All patients scored maximum points in language subtests of the ACE and the neurological examination did not reveal any pathological alterations in this function; therefore, deeper testing of language was not performed in this study.

Discussion

Here, we describe the neurocognitive assessment of the first Hungarian SCA28 family caused by a novel heterozygous missense mutation of the *AFG3L2* gene. The identified c.2011G>C nucleotide change is a novel variant determining the p.Gly671Arg alteration, which is pathogenic [8].

Table 2 demonstrates that the clinical phenotype of our patients is similar to that of the 82 earlier published SCA28 patients [6–8, 10–18]. The lack of ophthalmoparesis, ptosis, and slowing of saccades in our patients are the main differences, but these symptoms usually appear later in the course of

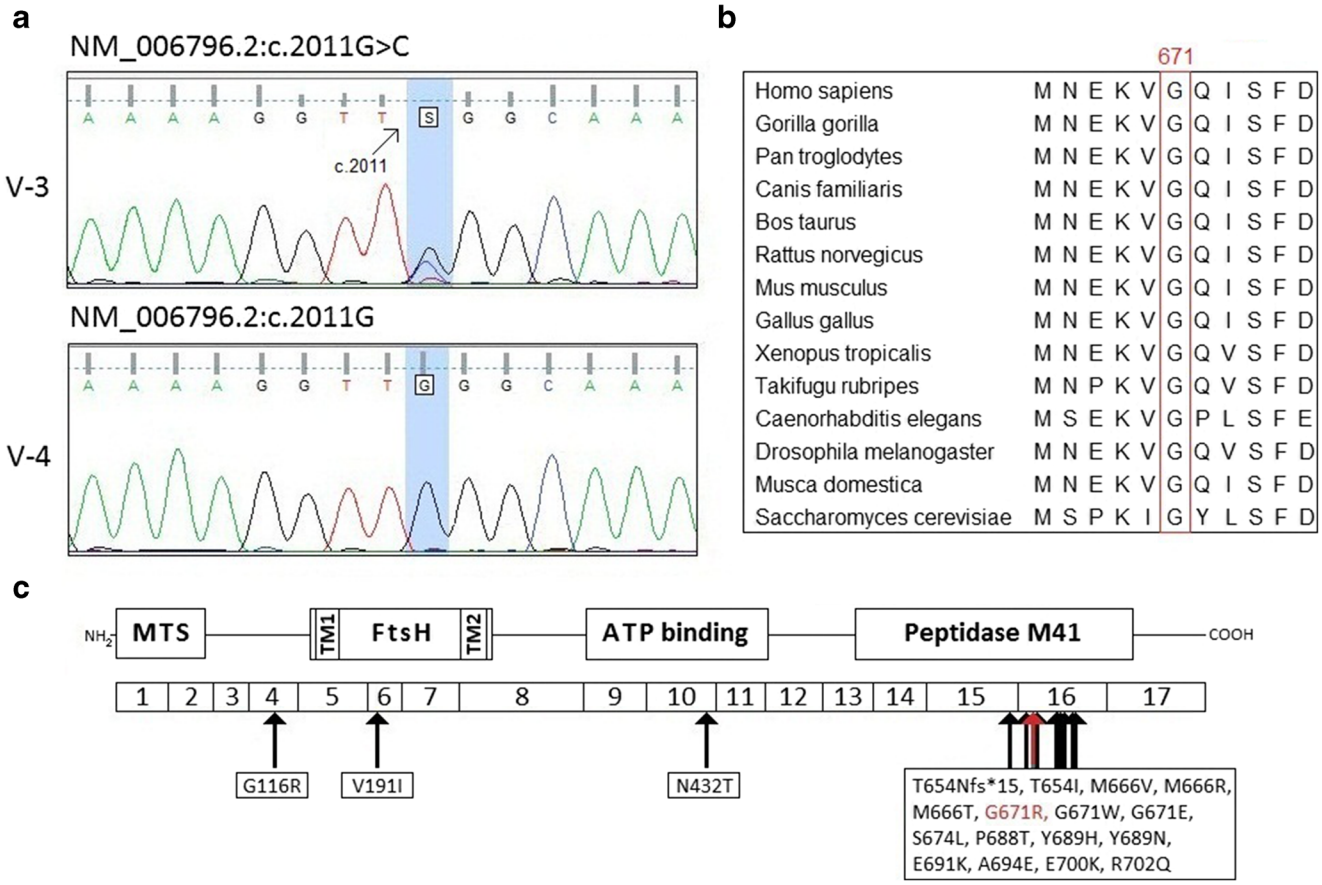

Fig. 3 **a** DNA sequence of the *AFG3L2* gene in patient 3 (*upper*: showing c.2011G>C heterozygous mutation) and in his healthy sister (*lower*: normal). **b** The identified p.Gly671Arg amino acid change is located within a highly conserved region of the protein. **c** *Upper*: structure of the AFG3L2 protein. *Lower*: exons and locations of the SCA28-causing mutations of the *AFG3L2* gene. *Red color*: the identified mutation variation in the Hungarian family

the disease. In our family, only patients 1 and 2 are older than 50 years, while the mean age of these ophthalmological signs of the previously reported SCA28 patients was 65.4, 57, and 55.4 years, respectively.

Beyond that all patients demonstrated normal cognitive performance globally, the psychological investigation revealed mildly lower capabilities in working memory, in visuospatial immediate memory and semantic memory, and in executive functions. These abnormalities are similar to the cognitive alterations that were found in SCA1 (executive dysfunction) and SCA2 and 3 (executive and visuospatial disabilities) [3, 4]. This may reflect the disturbance of the cerebellar-prefrontal connection system [32, 33] due to the presented cerebellar abnormalities. The cognitive deficits of our patients are similar to the most common SCAs and might be the part of the Cerebellar Cognitive and Affective Syndrome (CCAS), also known as Schmahmann's syndrome reported by Schmahmann and Sherman in 1998 [34]. This report described 20 patients (13 cerebellar stroke, three postinfectious cerebellitis, three cerebellar cortical atrophy, and one excision of a midline tumor) with CCAS [34]. Malm et al. also reported young adult patients (aged 18 to 44 years) with infratentorial infarcts having symptoms of the CCAS in 1998 [35]. Neau et al. described the same cognitive disturbances in elder patients (aged 39–75 years) with cerebellar infarcts in 2000 [36]. Fitzpatrick et al. investigated patients (mean age 53.41 years) with alcoholic cerebellar degeneration and delineated similar cognitive abnormalities in 2013 [37]. Schmahmann's syndrome may occur in children as well; Levisohn et al. and Riva et al. both published CCAS in children following resection of cerebellar tumors in 2000 [38, 39]. This syndrome is supposed to derive from the disruption of cerebellar neural circuits linking the cerebellum to prefrontal, posterior parietal, superior temporal, and limbic cortical areas, typically leading to deficits in executive and visuospatial functions, language, and emotional regulation [40]. There is a varying severity of the symptoms in patients with the same cerebellar pathology. This variability is mainly due to the location of the affected part of the cerebellum, because cognitive functions are supposed to be chiefly mediated by the posterior lobe and dentate nucleus, while affective modulation is thought to be found within the flocculonodular lobes, the posterior vermis, and the fastigial nucleus. In this context, our patients demonstrated only mild deficits in executive and visuospatial functions. We

Table 2 Comparison of our patients' clinical phenotype with 82 earlier-described SCA patients [6–8, 10–18]

		Data on 82 previously reported SCA28 patients	Our patients
Mean age at onset (years) (range)		30.88 (3–70)	18.2 (14–28)
Male/female gender		43/39	2/3
Gait ataxia	Mi	33/74	3/5
	Mo	32/74	2/5
	S	9/74	0/5
Limb ataxia	Mi	27/54	UL: 5/5; LL: 2/5
	Mo	24/54	UL: 0/5; LL: 2/5
	S	3/54	UL: 0/5; LL: 1/5
Dysarthria	Mi	30/63	3/5
	Mo	25/63	2/5
	S	8/63	0/5
Nystagmus		36/65	5/5
Ophthalmoparesis		38/70	0/5
Ptosis		30/58	0/5
Slowing of saccades		12/18	0/5
Brisk/increased tendon reflexes		47/69	3/5
Babinski sign		14/68	0/5
Spasticity		9/71	0/5

As the available data are limited from the literature, the numbers of affected individuals are presented out of the reported patients

Mi mild, *Mo* moderate, *S* severe, *UL* upper limbs, *LL* lower limbs

found no significant deficits in language functions, whereas emotional processing was not assessed in detail. Furthermore, it is essential to note that performance on the applied neuropsychological tests provided only indirect information about functioning of the related brain areas, because the applied tasks require the coordinated functioning of several interconnected pathways and networks. Although the difference in age of the examined subjects might introduce a greater variability to study results, the presentation of a single family may conversely decrease this variability by avoiding some interfamilial differences. Furthermore, normal values for the corresponding age groups are presented with cognitive findings, where available, thereby supporting the better comparison of results.

In conclusion, the novel c.2011G>C variation that was found in this Hungarian family demonstrated similar symptoms to previously reported cases. Reviewing the available data from the scientific literature, only 9 out of 82 were described to have some kind of cognitive abnormality, without further specification [6–8, 10–18]. Despite some limitations, including low case number and the fact that neuropsychological tests are poor localizers of brain pathology, the cognitive investigation performed in this study revealed slight abnormalities which may indicate the importance of integrity of cerebellar functioning in intact prefrontal activity [41]. These pilot findings may enhance the setup of better genotype-phenotype correlations in SCA28 and other SCAs, having future therapeutic aspects as well, keeping in mind a later expansion of this study, hopefully with a larger number of study population to be able to draw statistical conclusions as well.

Acknowledgements This work was supported by KTIA Grant No. 13 NAP-A-II/17. Denes Zadori was supported by the Janos Bolyai Research Scholarship of the Hungarian Academy of Sciences.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Ethical Approval Written informed consent was obtained from the patients for the publication of this study (institutional research committee registration number is 44/2016). All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent Informed consent was obtained from all individual participants included in the study.

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Neurological Sciences
https://doi.org/10.1007/s10072-019-04044-6

ORIGINAL ARTICLE

Predominant neurological phenotype in a Hungarian family with two novel mutations in the *XPA* gene—case series

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Received: 25 March 2019 / Accepted: 13 August 2019

Abstract

Objective The prevalence of xeroderma pigmentosum (XP) is quite low in Europe, which may result in a delay in determining the appropriate diagnosis. Furthermore, some subtypes of XP, including XPA, may manifest themselves with quite severe neurological symptoms in addition to the characteristic dermatological lesions. Accordingly, the aim of the current study is to highlight the predominant neurological aspects of XPA, as well as mild-to-moderate dermatological signs in a Hungarian family with 5 affected siblings.

Case reports The symptoms of the Caucasian male proband started to develop at 13–14 years of age with predominantly cerebellar, hippocampal, and brainstem alterations. His elder sister and three younger brothers all presented similar, but less expressed neurological signs. The diagnostic work-up, including clinical exome sequencing, revealed 2 novel compound heterozygous mutations (p.Gln146_Tyr148delinsHis, p.Arg258TyrfsTer5) in the *XPA* gene. Surprisingly, only mild-to-moderate dermatological alterations were observed, and less severe characteristic ophthalmological and auditory signs were detected.

Conclusions In summary, we present the first family with genetically confirmed XPA in the Central-Eastern region of Europe, clearly supporting the notion that disturbed function of the C-terminal region of the XPA protein contributes to the development of age-dependent neurologically predominant signs. This case series may help clinicians recognize this rare disorder.

Keywords Xeroderma pigmentosum group A · Neurological · Ataxia · Parkinsonism · Cognitive · Neuropathology

Introduction

Xeroderma pigmentosum (XP) is a rare autosomal recessively inherited condition with 100% penetrance [1] and only with a prevalence of 2.3 per million in Western Europe [2]. There are several distinct clinical subtypes of XP, including the variant form and those marked with letters A–G based on the different genetic cause. The characteristic cutaneous signs are exaggerated

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10072-019-04044-6) contains supplementary material, which is available to authorized users.

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Published online: 02 September 2019

sensitivity to sunlight, early development of freckle-like lentiginous pigmentation, and increased propensity for the formation of malignant skin tumors. The ocular alterations may involve conjunctival xerosis, corneal drying, and conjunctivitis. Neurological signs, such as intellectual disability, speech disturbance, sensorineural hearing loss, peripheral neuropathy, corticospinal alterations, and movement disorders with a predominant cerebellar ataxia resulting in severe walking disability, may also develop, especially in XPA [1, 3]. The aim of the current study is to present a family with 5 affected siblings diagnosed with novel compound heterozygous mutations in the XPA gene in accordance with the CARE (CAse REport) guidelines [4]. Additionally, this report is unique in its detailed genetical, neuropathological, ophthalmological, and dermatopathological assessment in addition to the comprehensive delineation of clinical symptoms and signs. Accordingly, this case series may add to the known phenotypic spectrum of XPA.

Case reports

Case histories, neurological, and related alterations

The Caucasian male proband (II-2 in Fig. 1), who died at 39 years of age, was first admitted to our clinic for a diagnostic work-up of his unknown cognitive and movement disorder at 36 years of age. In addition to the presence of a slightly exaggerated sunburn reaction, his neurological symptoms started to develop at his 13–14 years of age. His speech became slurred and his cognitive functions deteriorated as well, resulting in progressive and severe learning disabilities. He completed 11 classes and later he worked in a twine factory until his 26 years of age, and then his disability led to retirement, and he became dependent on his parents. Repeated falls occurred with scarring and his swallowing functions and vision deteriorated as well.

Upon neurological examination, he presented signs of disturbed eye movements (exophoria, restricted eye movements in all directions with diplopia, gaze-evoked nystagmus), dysarthria, hypo-/areflexia, pathological reflexes, and decreased sense of vibration. Furthermore, movement disorder with dominating ataxia and parkinsonism (bilateral dysmetria, cerebellar predominant mixed limb ataxia more pronounced in the legs, truncal ataxia, severe postural instability, broad-based, ataxic gait, moderate, mainly left-sided bradykinesia and upper limb dystonia, mild rigidity on provocation, mild postural tremor, and occasional myoclonic jerks) was also detected (Supplementary Material 1.1 and 2). The myoclonic jerks were spontaneous, distal, and as they can be exacerbated by sensory stimuli, they were presumed to be cortical. The neuropsychological assessment revealed the signs of severe cognitive dysfunction confined to two functional neuroanatomical networks, the hippocampus-dependent and that related to the prefronto-cerebellar system, with similar degrees of impairment (Supplementary Material 1.2).

The brain MRI revealed pronounced generalized atrophy with slight predominance regarding the parieto-occipital and cerebellar structures (Supplementary Material 1.3). The degree of atrophy seemed to correlate with the severity of neurological signs.

Electroneurography demonstrated mixed sensorimotor lower limb predominant polyneuropathy.

Following his death caused by aspiration pneumonia resulting from dysphagia as a part of progressive neurological impairments, comprehensive post mortem neuropathological assessment was carried out. The most prominent alterations demonstrated by that were asymmetrical hippocampal sclerosis and Purkinje cell degeneration along with moderate loss of neurons in the substantia nigra and a scattered infiltration of CD8-positive T lymphocytes (for details, please see Supplementary Material 1.4 and 1.5).

Regarding the family history of the proband, the presence of similar, but less expressed, deterioration was identified in his sister (II-1, 3 years older than the proband—Supplementary Materials 1.6 and 3), and his three brothers (II-3, 3 years younger than the proband—Supplementary Materials 1.7 and 4; II-4, 5 years younger than the proband—Supplementary Materials 1.8 and 5; II-5, 14 years

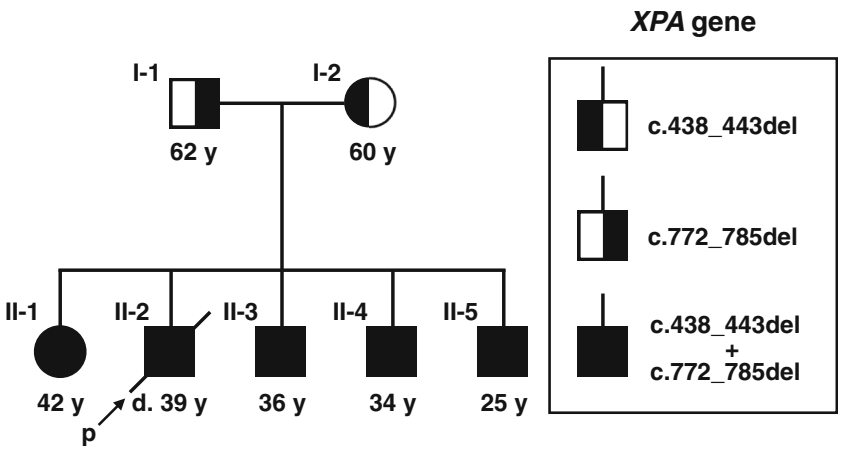

Fig. 1 Pedigree of the assessed family with mutation in the *XPA* gene

younger than the proband—Supplementary Materials 1.9 and 6), respectively (Fig. 1). Besides a mild light sensitivity in the father (I-1), there were no major relevant symptoms in the parents; the demonstrated slight cognitive impairment may have other explanations unrelated to XPA (Supplementary Material 1.2).

Dermatological and dermatopathological assessments

Dermatological alterations generally include mild-to-moderate solar damage on sun-exposed areas with the presence of hyperpigmentation in the basal keratinocytes and solid and infiltrative basal cell carcinomas (Supplementary Material 1.10).

The results of the neurological and dermatological examinations enabled staging of all 5 siblings according to the Japanese Dermatological Association's guideline (Table 1; [3]).

Ophthalmological assessment

Ophthalmological examinations demonstrated exophoria and weakness of convergence with gaze-dependent diplopia in patients II-1 and II-2. Pathological alterations of the vitreo-macular interface, i.e., thickened internal limiting membrane (ILM) with ruffled inner surface in the macular area of the retina, were diagnosed in patients II-2, II-3, and II-4. This sign can be considered as a gliotic proliferation of the inner surface of the ILM, although epiretinal membrane formation could not be detected.

Diagnostic challenges and genomic studies

The major diagnostic challenges were that besides the severe neurological symptoms and signs, the dermatological alterations were not so prominent to be easily recognized by a neurologist.

Variants obtained by exome sequencing (for details, please see Supplementary Material 1.11) were filtered based on severity and frequency against public variant databases including dbSNP, ClinVar, ExAC, EVS, GnomAD, and an in-house clinical exome database of 300 unrelated Hungarian samples.

For the two new XPA variants (NM_000380.3:c.438_443delAGAATA; NP_000371.1:p.Gln146_Tyr148delinsHis and NM_000380.3:c.772_785delCGTAAGACTTGTAC; NP_000371.1:p.Arg258TyrfsTer5), ClinVar accessions VCV000523609.1 and VCV000523609.1 were assigned, respectively. The minor allele frequency (MAF) for *XPA* c.438_443delAGAATA variant is unknown since it is not listed in either the gnomAD (https://gnomad.broadinstitute.org/), ExAc (http://exac.broadinstitute.org/), or in the EVS (https://evs.gs.washington.edu/EVS/) databases. The MAF for *XPA* c.772_785delCGTAAGACTTGTAC variant is 0.0000814 (23/282538 allele) according to the gnomAD (https://gnomad.broadinstitute.org/) database, which is within the range for a pathogenic recessive allele. Based on the American College of Medical Genetics and Genomics variant interpretation guidelines [5], the first variant was classified as likely pathogenic, whereas the second as pathogenic. The conservation of the region of XPA protein affected by the in-frame mutation is demonstrated by Supplementary Material 1.12.

Discussion

Regarding genotype-phenotype correlation, mutations affecting exons 2, 3 and introns 1, 3 in the *XPA* gene are almost

Table 1 The staging of XPA patients according to the classification of the severity of XP proposed by the Japanese Dermatological Association's guidelines [3]

	Patient II-1	Patient II-2	Patient II-3	Patient II-4	Patient II-5
Cutaneous symptom (D) score					
Exaggerated sunburn	3	3	3	3	3
Freckle-like eruption	3	3	1	2	0
Skin cancer	3	2	0	0	0
Severity of cutaneous symptoms	D3	D3	D2	D2	D2
Extracutaneous symptom (N) score					
Hearing ability	1	N.A.	1	0	0
Movement	2	3	2	2	2
Intellectual functions	2	3	2	2	2
Swallowing and respiratory function	0	3	0	0	0
Severity of extracutaneous symptoms	N3	N3	N3	N2	N2
Classification of XP depending on the severity					
Stage	4	4	4	3	3

XP, xeroderma pigmentosum; *N.A.*, not available

always accompanied by severe characteristic clinical signs, whereas mutation sites approaching the C-terminal of the corresponding XPA protein may be considered as hypomorphic, i.e., they can be characterized by less severe neurological and especially, dermatological phenotypes [6, 7] (the clinical and demographic characteristics of all the published mutations in the *XPA* gene were collected and outlined in Supplementary Material 1.13). Indeed, cutaneous signs may be absent as well [8]. In addition to the mutation site, the clinical phenotype may be influenced by the age of the patient and, for dermatological and ophthalmological alterations, by sun exposure as well, even within the same family [9].

This genotype-phenotype correlation was confirmed by the current study as well, i.e., only mild-to-moderate dermatological and no prominent ophthalmological and audiological, but severe neurological signs evolved with age.

Despite the hypomorphic character of the reported variants, the likely pathogenic feature of the in-frame mutation in exon 4 (p.Gln146_Tyr148delinsHis) is supported by that it affects a conserved region of the protein (presented in Supplementary Material 1.12) including p.Glu147 and p.Tyr148, the deletion of which results in the pathologically reduced binding of XPA protein to replication protein A [10]. The second one in exon 6 (p.Arg258TyrfsTer5) may be considered pathogenic as it eventuates a premature stop codon. In addition to its frameshift character, the pathogenicity of this variant may be further supported by a later unrelated report of it in a patient with XPA (https://www.ncbi.nlm.nih.gov/clinvar/variation/523608/).

Furthermore, in addition to the presentation of a very detailed phenotypic description from a neurological point of view, the present study yields several novelties: (1) 5 out of 5 affected siblings within a single family has not been reported before, and this study presents the first cases of XPA in the Central-Eastern region of Europe. (2) Likely pathogenic novel in-frame and pathogenic novel frameshift mutations in trans position are presented. (3) A previously unreported scattered infiltration of CD8+ T lymphocytes was detected in the proband with XPA, without a manifest corresponding dermatological alteration. In light of the fact that in common neurodegenerative diseases with accumulation of proteins, CD8+ cytotoxic T cells are not considered as hallmark lesions; this finding is unexpected and may be part of an immunological activation following an insult from a currently uncharacterized agent or process. However, the significance of this observation could not be clarified in the present study and merits further observations on similar cases.

In conclusion, the presentation of this unusual neurological predominant phenotype of XPA may help clinicians recognize this rare disorder.

Acknowledgements Open access funding provided by University of Szeged (SZTE).

Funding information This study was supported by the GINOP-2.3.2-15-2 grant (TK, ZM), by the GINOP-2.3.2-15-2016-00034 and 20391-3/2018/FEKUSTRAT grants (LV), by the OTKA125509 and GINOP-2.3.2-15-2016-00020 grants (IBN), and by the National Brain Research Program 2017-1.2.1-NKP-2017-00002_VI/4 (PK, LV) provided by The National Research, Development and Innovation Office (Hungary). The state funders played no role in the study design, data collection or analysis, decision to publish, or in the preparation of the manuscript. DZ and IBN were supported by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences.

Compliance with ethical standards

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

Ethical approval Written informed consent was obtained from all individual participants included in the study. Additional informed consent was obtained from all individual participants for whom identifying information is included in this article (Regional Human Biomedical Research Ethics Committee of the University of Szeged registration numbers are 150/2014. and 44/2016., respectively). All procedures performed in studies involving human participants were in accordance with the ethical standards of the Regional Human Biomedical Research Ethics Committee of the University of Szeged and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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VI.

BMC Neuroscience

RESEARCH ARTICLE

Open Access

Eye-tracking-aided characterization of saccades and antisaccades in *SYNE1* ataxia patients: a pilot study

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Abstract

Background: *SYNE1* ataxia is an autosomal recessive hereditary condition, the main characteristic features of which are gait and limb ataxia and cerebellar dysarthria. Reports have revealed that the clinical phenotype of *SYNE1* ataxia is more complex than the first published cases with pure cerebellar signs indicated. The aim of this study was to characterize eye movement alterations in the first diagnosed Hungarian *SYNE1* ataxia patients.

Results: Saccades and antisaccades were examined with an eye tracker device in 3 *SYNE1* (one patient has two frameshift mutations [c.8515_8516insA, p.Met2839Asnfs*53 and c.11594_11595insG, p.Glu3866*] in a compound heterozygous state, whereas two subjects have a splicing variant [c.23146-2A > G] in a homozygous state), 6 Friedreich ataxia (FA) patients and 12 healthy controls. Besides that, detailed clinical phenotyping and comprehensive neuropsychological assessment were carried out in all patients with ataxia.

In addition to the characteristic cerebellar alterations, pyramidal signs and polyneuropathy were observed at least in 2 *SYNE1* ataxia patients, for which no other underlying reason was found. The eye tracking assessment revealed hypometric saccades in the longer amplitude (18.4°) saccadic paradigm in all *SYNE1* patients, whereas 2 out of 3 *SYNE1* subjects performed slow saccades as well. In the antisaccade task, higher incorrect ratios of antisaccades were demonstrated in *SYNE1* patients compared to healthy controls, showing inverse correlation with working memory test results. The corresponding data of FA patients was dispersed over a wide range, partially overlapping with control data.

Conclusions: The current study draws attention to the presence of eye movement disorders in patients with *SYNE1* ataxia and demonstrates that alterations in the antisaccade paradigm may be related to working memory deficits.

Keywords: *SYNE1*, Ataxia, Genetics, Eye movement, Eye tracking, Saccade

Background

Autosomal recessive cerebellar ataxias (ARCA) belong to a continuously expanding group of hereditary neurodegenerative disorders. Recently, more than 100 genes have been identified which can cause ARCA, including the *SYNE1* gene (OMIM 608,441). *SYNE1* is one of the largest genes in the human genome, located in 6p25 chromosome and containing 146 exons [1]. This huge

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gene encodes a peptide of about 8797 amino acids, known as Nesprin 1 (Nuclear envelope spectrin 1) [1]. It is a member of the spectrin family of proteins and its major function is to link the plasma membrane to the actin cytoskeleton [2]. Nesprin 1 has three domains, including the N-terminal actin binding domain (also called calponin homology domain), multiple spectrin repeats and the C-terminal KASH domain (also knowns as Klarsicht domain) [1]. In 2007, Gros-Louis et al. reported 26 French-Canadian families from Quebec, Canada with slowly progressive pure cerebellar hereditary ataxia caused by truncating mutations of the *SYNE1* gene. The name of this disease was autosomal recessive cerebellar ataxia type 1 (ARCA1), also known as spinocerebellar ataxia, autosomal recessive 8 (SCAR8), or recessive ataxia of Beauce [1]. In the following years, *SYNE1* ataxia was observed almost exclusively in Quebec, Canada [1, 3]. From 2013, some sporadic cases were reported outside the French-Canadian population as well [4–7]. In 2016, Synofzik and Mademan et al. described 33 non-Canadian patients with *SYNE1* ataxia from a large multi-center study, which indicated that mutations of *SYNE1* gene are much more common causes of ARCA than previously thought [2, 8]. Besides its frequency, the clinical phenotype was also more complex than the first described, purely cerebellar disease. Most of the newly identified patients had extracerebellar neurological signs, including upper and lower motoneuron symptoms, and non-neurological abnormalities, including scoliosis, pes cavus or respiratory dysfunction with severe manifestation. Only a small portion of these subjects showed the classical pure cerebellar phenotype [2, 8].

Moreover, mutations of *SYNE1* gene have been associated with arthrogryposis multiplex congenita, Emery-Dreifuss muscular dystrophy 4, dilatative and hypertrophic cardiomyopathy, intellectual disability, blepharospasm, autism spectrum disorder and schizophrenia [9–16].

After reviewing the clinical phenotype of the previously published 168 *SYNE1* ataxia patients, it was noted that detailed characterization of eye movements had not yet been performed, only the occurrence of gaze-evoked nystagmus, slowing of saccades, broken up smooth pursuits, strabismus and square-wave jerks were reported [1–4, 17–19].

In this paper we aimed to characterize the saccadic and antisaccadic eye movements of 3 Hungarian *SYNE1* ataxia patients and compare them to the same parameters of Friedreich ataxia (FA) patients and healthy subjects in addition to detailed clinical phenotyping and comprehensive neuropsychological assessment.

Patients and methods

Participants

9 patients with unknown cerebellar ataxia and 12 healthy controls (HC) were enrolled in the study. The patients underwent a detailed diagnostic approach including neurological examination, laboratory and radiological investigations to exclude acquired causes of ataxia. Scale for the Assessment and Rating of Ataxia (SARA) scores were recorded in all cases. After obtaining written, informed consent, genomic DNA was extracted from peripheral blood leukocytes by standard protocol. First, according to recent guidelines on the management of sporadic ataxias without known secondary etiology [20], the most common repeat expansion hereditary ataxias (spinocerebellar ataxia (SCA) 1, 2, 3, 6, 7 and FA) were tested. If these genetic tests did not confirm the diagnosis, new generation sequencing (NGS) was performed.

For proband AT-04, whole exome sequencing (WES) was performed with SureSelectXT Human kit All Exon v7 (Agilent, Agilent Technologies, Santa Clara, CA) according to the manufacturer's instructions and paired-end sequenced (2×100 bp) on HiSeq 1500 (Illumina, San Diego, CA, USA). Prioritized variants were validated in the proband, in the parents of the proband and in his brother by amplicon deep sequencing performed using Nextera XT Kit (Illumina) and sequenced on HiSeq 1500 (Illumina).

For subjects AT-05 and AT-06, a total of 60 ng of genomic DNA was used for library preparation and sequenced with Trusight One clinical exome kit (Illumina) on Illumina MiSeq platform. The clinical exome kit covers the coding region of 4813 clinically relevant, disease-associated genes. The 150 bp paired reads were aligned to the GRCh37.75 human reference genome by Burrows Wheel Aligner (BWA v0.7.9a) software. The variants were called by Genome Analysis Toolkit HaplotypeCaller (GATK v3.5) best practice; annotated by SnpEff and VariantStudio softwares. Variants were filtered based on severity and frequency against public variant databases, including dbSNP, ClinVar, ExAC, EVS and an in-house clinical exome database of 140 unrelated Hungarian patients.

Eye tracking

Recording system

The system and paradigm that were used are described in a previous study [21]. The assessment was performed in a well-lit room. Subjects sat in front of the monitor and their heads were fixed at a distance 60 cm from the screen. We used a Tobii TX300 eye tracker and tasks were programmed in Psychophysics Toolbox V 3.0.12,

under MatLab. Before every paradigm, a five-points calibration was performed.

Saccade task

Subjects accomplished the following visually guided saccade task: a black cross appeared at the center of the screen and 1.2–2 s later it jumped to the right or left side of the screen. The background was grey and the distances of displacement of the cross were 9.2° or 18.4° horizontally. All measurements were repeated 20 times in a pseudorandom order, this means 80 measurements per subject. The participants had to shift their gaze to the new position of the target as fast and accurately as they could. There was a break half-way through the task to prevent subjects tearing and/or tiring.

Antisaccade task

In the antisaccade task, the simple antisaccade paradigm was used [22]. The composition was similar to the visually guided saccade paradigm, however, the participants had to direct their gaze in the opposite direction (e.g. if the target appeared on the left side, they had to look to the right side). We explained explicitly the antisaccade paradigm to the patients before the task and answered their questions. We particularly highlighted for the participants that the antisaccade task needs more attention. Before the trial, all patients confirmed that they understood the task instructions. Only horizontal movements were recorded, as in the saccade task. There was also a break after the first half of the trial.

Data acquisition and processing

Data recording began when the target jumped to the periphery and stayed there for one second. The recording frequency was 300 Hz and both eyes were registered separately. We used a semi-automatic, in-house script to define parameters of saccades, as described in a previous study [21]. The following parameters were measured: peak velocity, latency, amplitude, gain and duration. In the saccade task, we assessed the main sequence relationships of duration versus amplitude and peak velocity versus amplitude using the linear model [23]. Additionally, in the antisaccade paradigm the incorrect ratio of antisaccades was also examined. It is a quotient showing the incorrectly executed antisaccades, calculated as incorrect/(incorrect + correct) antisaccades.

Neuropsychological assessment

The enrolled ataxia patients were assessed via cognitive examination performed by trained neuropsychologists. The global cognitive performance was measured by Addenbrooke's Cognitive Examination (ACE) including the Mini-Mental State Examination (MMSE). Executive function was evaluated by verbal and semantic fluency tests. In addition, working memory and the ability to maintain and manipulate information were estimated by the Backward Digit Span Task (BDST) and the Listening Span Task (LST). The quality of information planning and visuo-constructional and visual organizational abilities were assessed by the Rey Complex Figure Test (RCFT).

Results

Patients

The repeat expansion examinations verified the FA diagnosis of 6 patients. All of them had homozygous GAA repeat expansions in the first intron of the *FXN* gene. The remaining three patients had negative repeat expansion tests, therefore NGS was performed and it confirmed *SYNE1* gene abnormalities. The mean age of FA patients and HC group participants was the same, and the three *SYNE1* patients were in a similar age range. The demographic and clinical data of FA and *SYNE1* patients and healthy subjects are summarized in Table 1, while the thorough clinical and genetic characteristics of *SYNE1* patients are detailed here. AT-04 subject was the second child of Hungarian, non-consanguineous parents. There was no neurological disease in his family. His first symptom was gait ataxia at the age of 15 years. He also had delayed puberty in this period. Later, slurred speech also appeared and his gait imbalance progressed. The neurological examination revealed gaze-evoked horizontal nystagmus, cerebellar dysarthria, bilateral Babinski sign, gait ataxia and severe lower limb ataxia and mild numbness in the upper extremities. Sometimes stimulus sensitive myoclonic jerks could also be observed. He had strabismus and myopia with negative fundoscopy. Electroneurography showed mild axonal sensory polyneuropathy. Currently, the patient requires walking sticks because of the progression of his symptoms. Laboratory examination did not find pathological abnormalities. Brain MRI was performed after sixteen years of disease course and displayed moderate cerebellar atrophy with preserved brainstem and supratentorial structures (Fig. 1a, b).

WES of AT-04 patient revealed a compound heterozygote state in *SYNE1* gene NM_033071.3:c.8515_8516insA, p.Met2839Asnfs*53 and NM_033071.3:c.11594_11595insG, p.Glu3866* (Fig. 2a). The c.8515_8516insA variant located in exon 55 out of 146 was inherited from the mother of the proband, while c.11594_11595insG located in exon 71 was inherited from the father, and both variants were absent in the healthy brother of the proband. None of the frameshift variants were found in the gnomAD database (www.gnomad.broadinstitute.org) and they are predicted to cause the loss of the full-length SYNE1 protein (8750 amino acids).

Table 1 Demographic, clinical and genetic data of *SYNE1* and FA ataxia patients and healthy controls

Patient code/ Group name	Age (years)	Sex	Mutation (cDNA)	Protein change or variant type	Age at onset (years)	Gait ataxia	Upper limb ataxia	Lower limb ataxia	Dys-arthria	GEN	UMN	LMN	PNP	SARA	Brain MRI	Other sign/ disease
AT-04	Mean: 38.3 ± 3.40 (35–43)	M	c.8515_8516insA c.11594_11595_insG	p.Met2839Asnfs*53 p.Glu3866*	15	+++	++	+++	++	Y	Y	N	Mild ASN	23.5	Cerebellar atrophy	Delayed puberty, myoclonic jerks, myopia, strabism
AT-05		F	c.23146-2A>G	Splicing	30	+++	++	+++	++	N	Y	N	N	25	Cerebellar and cerebral cortical atrophy	DM, HC, HT, obesity
AT-06		F	c.23146-2A>G	Splicing	14	++	+	+	+	N	Y	N	MSMN	12	Cerebellar and cerebral cortical atrophy	DM, HC, HT, obesity, pes cavus
FA group	Mean: 41.5 ± 17.97 (16–60)	3 M, 3F	Homozygous intronic GAA repeat repeat expansion	Decreased frataxin expression	Mean: 25.83 ± 16.64 (7–49)	++5/6 +++1/6	+4/6 ++2/6	+1/6 ++4/6 +++1/6	+1/6 ++4/6 +++1/6	N 6/6	Y 5/6 N 1/6	Y 1/6 N 5/6	ASN 2/6 ASMN 1/6 NSP 2/6 N 1/6	Mean: 16 ± 6.5 (13–30.5)	–	–
HC group (ST)	Mean: 40.0 ± 10.58 (28–59)	4 M, 8F	–	–												
HC group (AST)	Mean: 40.25 ± 10.39 (28–59)	4 M, 8F	–	–												

+: mild, ++: moderate: +++: severe

ASMN axonal sensorimotor polyneuropathy, *ASN* axonal sensory polyneuropathy, *AST* antisaccade task, *DM* diabetes mellitus, *F* female, *GEN* gaze-evoked nystagmus, *HC* hypercholesterolemia, *HT* hypertension, *M* male, *MSMN* mixed sensorimotor polyneuropathy, *N* not present, *NSP* not specified polyneuropathy, *PNP* polyneuropathy, *SARA* Scale for the Assessment and Rating of Ataxia, *ST* saccades task, *UMN* upper motor neuron involvement, *Y* present

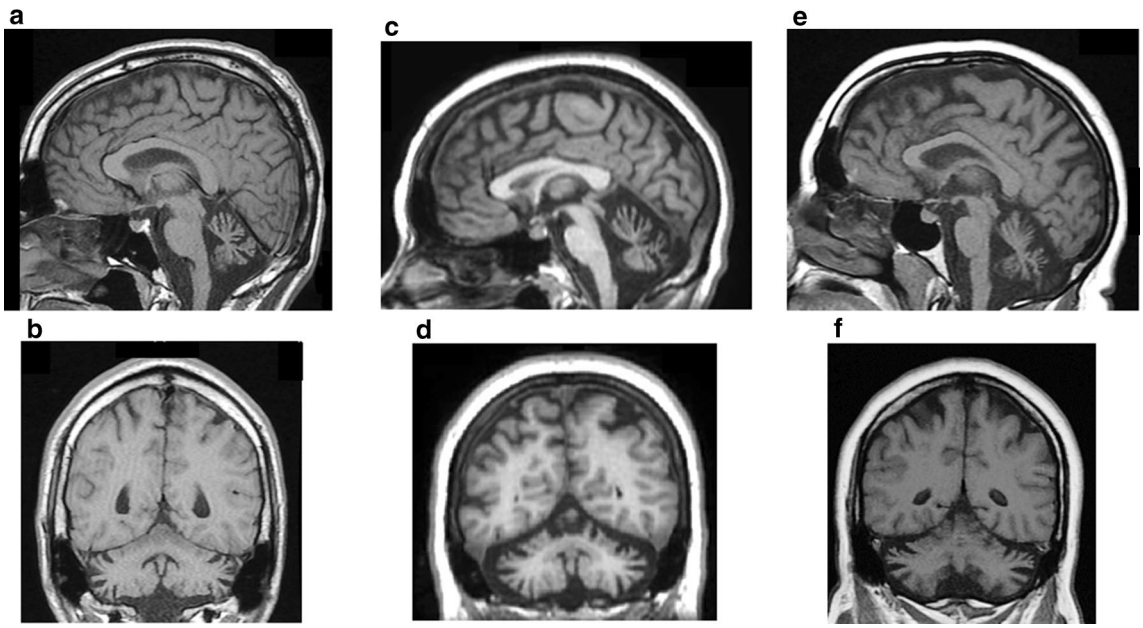

Fig. 1 Brain MRI scans of *SYNE1* ataxia patients demonstrated moderate cerebellar atrophy in all subjects and mild cortical atrophy in AT-05 and AT-06 patients. **a**, **b**: AT-04 patient; **c**, **d**: AT-05 patient; **e**, **f**: AT-06 patient; **a**, **c**, **e**: sagittal T1 weighted scans; **b**, **d**, **f**: coronal T1-weighted scans

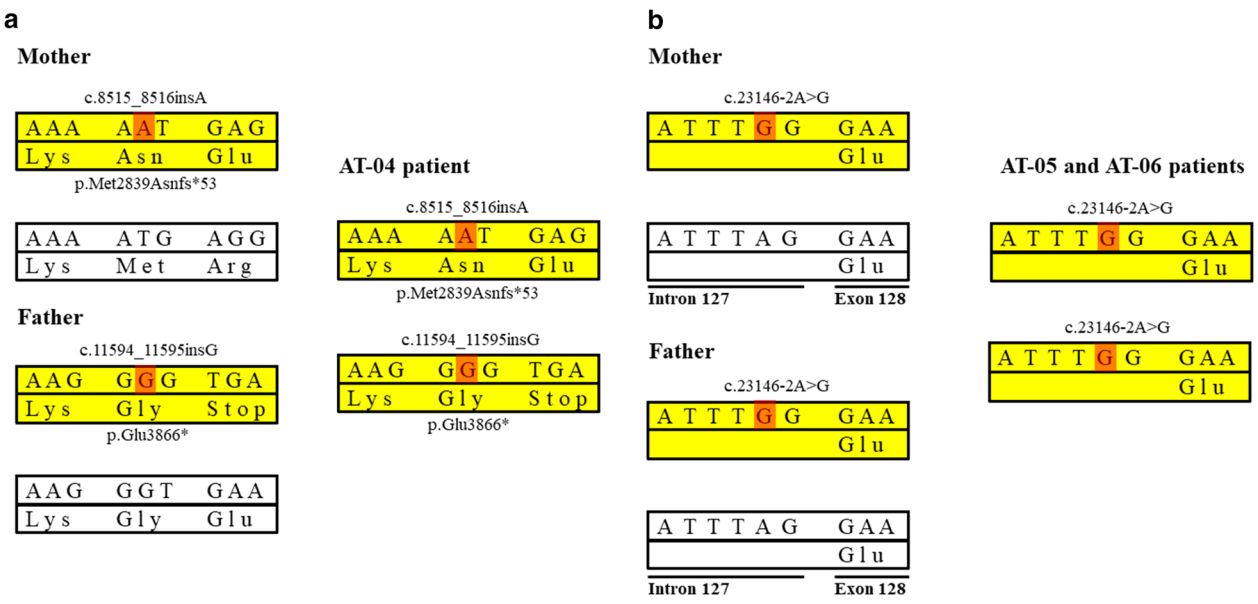

Fig. 2 Genetic abnormalities and consequent alterations of protein of *SYNE1* ataxia patients and their parents. **a**: *SYNE1* gene mutations in AT-04 patient and the parental origin of these variations. **b**: *SYNE1* gene abnormalities in AT-05 and AT-06 subjects and the parental segregation of these mutations. The upper parts of the bars denote the DNA sequence, while the lower parts show the encoded amino acids of the protein. Yellow bars indicate the pathogenic alleles, white bands mark the normal alleles. Red highlights the nucleotide change of the *SYNE1* gene. In part (**b**), the c.23146-2A > G mutation is located in the intron–exon boundary resulting in an abnormal splicing variant

The age at onset of AT-05 patient was 30 years and her first complaint was gait ataxia, whereas the first symptom of her sister (patient AT-06) appeared at 14 years of age and was gait abnormality as well. The neurological examination of both patients revealed cerebellar dysarthria and brisk tendon reflexes with bilateral Babinski signs.

Table 2 Saccade examination in *SYNE1* (AT-04–06) and Friedreich ataxia patients and in healthy controls

Subjects	9.2° saccades					18.4° saccades				
	Peak velocity (°/s)	Latency (s)	Amplitude (°)	Duration (s)	Gain	Peak velocity (°/s)	Latency (s)	Amplitude (°)	Duration (s)	Gain
AT-04	343.18	0.16	10.27	0.069	1.117	384.95	0.19	13.87	0.079	0.754
AT-05	219.14	0.27	7.434	0.076	0.808	279.08	0.27	11.48	0.087	0.624
AT-06	215.87	0.18	7.02	0.073	0.763	280.26	0.22	15.16	0.104	0.824
Median FA (range)	316.45 (264.62–382.63)	0.20 (0.18–0.31)	9.18 (7.86–11.83)	0.071 (0.066–0.084)	0.998 (0.855–1.285)	431.13 (326.02–555.22)	0.23 (0.21–0.34)	16.49 (14.35–20.85)	0.088 (0.083–0.104)	0.896 (0.780–1.133)
Median HC (range)	269.20 (233.54–333.55)	0.18 (0.17–0.21)	8.45 (7.99–9.21)	0.069 (0.059–0.079)	0.919 (0.869–1.001)	363.93 (321.89–505.69)	0.20 (0.17–0.26)	16.75 (15.12–17.29)	0.091 (0.075–0.102)	0.911 (0.822–0.940)

FA Friedreich ataxia, *HC* healthy control

Truncal ataxia was moderate in the younger patient (AT-06) and was severe in the elder subject (AT-05). After eleven years of disease course patient AT-05 could only walk with aids. Mild upper limb and moderate lower extremity incoordination developed in the younger sister, whereas her sibling had moderate superior and severe inferior limb ataxia. AT-05 patient has obesity, diabetes mellitus, hypertension and hypercholesterolemia, but ophthalmological and cardiological investigations were normal. AT-06 patient also has the same metabolic disorders, moreover, she has an excavated foot and electroneurography delineated multifocal sensorimotor mixed type polyneuropathy. The brain MRI showed moderate cerebellar and very mild cerebral cortical atrophy in both patients (Fig. 1c–f). Their non-consanguineous parents did not suffer from ataxia and the younger patient has two healthy children.

In AT-05 and AT-06 patients the same homozygous NM_182961.3:c.23146-2A > G alteration of the *SYNE1* gene was detected. This intronic variant was not found in gnomAD. It causes an A > G change at the Intron 127 – Exon 128 boundary resulting in an abnormal splicing variant (Fig. 2b). The presence of these mutations was confirmed by targeted Sanger sequencing. Segregation analysis identified this variant in the heterozygous state in both parents of the patients.

Eye tracking

Saccades

The pooled data of leftward and rightward saccades were analyzed (Table 2). There was not any relevant difference between the three groups of examined subjects in saccadic latencies and durations for either the shorter (9.2°) or the longer (18.4°) saccade paradigms. The peak velocities of saccades of AT-05 and AT-06 patients were smaller than the HC subjects and FA patients. However, the peak velocities of the saccades of AT-04 patient were similar to the subjects of HC and FA groups. In the 9.2° saccade task, AT-04 patient demonstrated hypermetric saccadic eye movements, whereas the other two *SYNE1* ataxia patients showed hypometric saccades. Nevertheless, in the 18.4° saccade task *SYNE1* ataxia subjects performed smaller saccadic amplitudes and gain than the healthy controls with minimal overlap (Fig. 3a). The amplitudes and gain of saccades of FA patients were in a similar range to that of the HC group. Figure 4 displays the main sequence relationships using the linear model. The duration vs. amplitude diagram (Fig. 4a) shows that saccades of *SYNE1* ataxia patients are hypometric and their duration is longer than in FA or HC groups. The peak velocity vs. amplitude graph (Fig. 4b) reinforces that the saccades of *SYNE1* patients are hypometric and their peak velocity is smaller than in HC or FA groups.

Antisaccades

The pooled data of leftward and rightward antisaccades were evaluated as well (Table 3). There was no remarkable difference between the groups with regard to peak velocities, latencies and durations of antisaccades. The incorrect ratios were higher in the *SYNE1* and FA patients than in the HC group. However, there was a mildly overlapping range in the 9.2° antisaccades within the *SYNE1* and HC subjects, whereas this was only minimally detected in the longer antisaccades (Fig. 3b).

Neuropsychological assessment

The neuropsychological assessment of FA and *SYNE1* patients are summarized in Table 4. The cognitive performance of ataxia patients was compared with the data of age- and education-matched standards in the literature [24–26]. Global cognition was only mildly reduced in two FA patients (AT-11 and AT-20), whereas the other subjects demonstrated normal ACE and MMSE scores. The LST results showed mild abnormalities in all *SYNE1* patients and in one FA patient, whereas the BDST results were decreased more prominently in both patient groups. These alterations indicate the impairment of working memory and in the ability to maintain and manipulate information. Surprisingly, the fluency test scores were in the normal range, only AT-04 patient demonstrated a mild deficit in the verbal fluency test. In addition, the RCFT results were equal to the standard outcomes, only AT-05 patient showed a mild impairment.

Discussion

In this paper we describe the clinical phenotype and characteristics of saccades and antisaccades of the first genetically confirmed Hungarian *SYNE1* patients caused by novel mutations. The cerebellar symptoms of these patients involved moderate to severe gait and lower limb ataxia and mild to moderate upper limb ataxia and dysarthria. Extracerebellar involvement was present as well, as all subjects have pyramidal signs and two of the three patients have some types of polyneuropathy. Moreover, AT-04 patient had strabismus, tactile sensitive myoclonic jerks and delayed puberty. In summary, the clinical phenotype of subjects is not purely cerebellar, in contrast to that described in the first French-Canadian population by Gros-Louis et al. [1], and similar to that of the later reported cases [2, 8]. This symptomatic variability suggests that *SYNE1* gene plays a broader role in the normal functioning of the nervous and musculoskeletal systems. Consequently, the mutations of this gene can cause symptoms and signs over a large spectrum, but

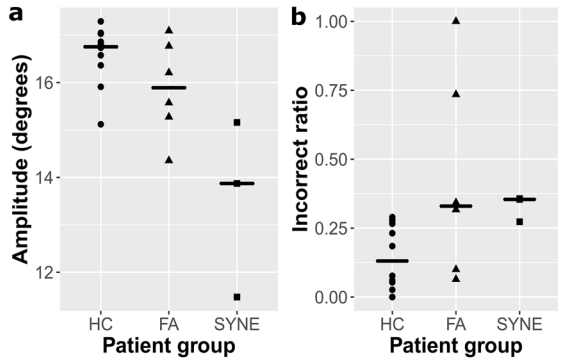

Fig. 3 The most characteristic alterations in saccadic and non-saccadic paradigms in *SYNE1* ataxia patients. **a**: saccadic amplitude of the 18.4 saccade paradigm in the different groups; **b**: incorrect ratios of the 18.4 antisaccade task in the investigated subjects; the circles, triangles and squares denote the parameters of healthy controls (HC), Friedreich ataxia patients (FA) and *SYNE1* patients, respectively, and the median values are demonstrated as well

an obvious genotype–phenotype correlation cannot be established [2].

The eye tracking examination revealed hypometric saccades in the 18.4° paradigm in all *SYNE1* patients and in two out of three in the 9.2° task. Saccadic dysmetria is a cerebellar symptom and it is a common eye movement abnormality in hereditary ataxias [27]. This is not a specific symptom for any type of inheritable ataxia, but there may be a higher proportion of hypo- or hypermetric saccades, serving as a supporting feature of the disease. The hypometria of *SYNE1* patients at large amplitude stimulus is more pronounced than the well-known mild hypometria in healthy subjects observed at higher target eccentricities [22]. The hypometria of *SYNE1* patients is presumably due to the involvement of the cerebellar oculomotor vermis and caudal fastigial nucleus [28]. In addition to accuracy, velocity is another important characteristic of saccades. Previous case reports described slowing of saccades in a portion of *SYNE1*

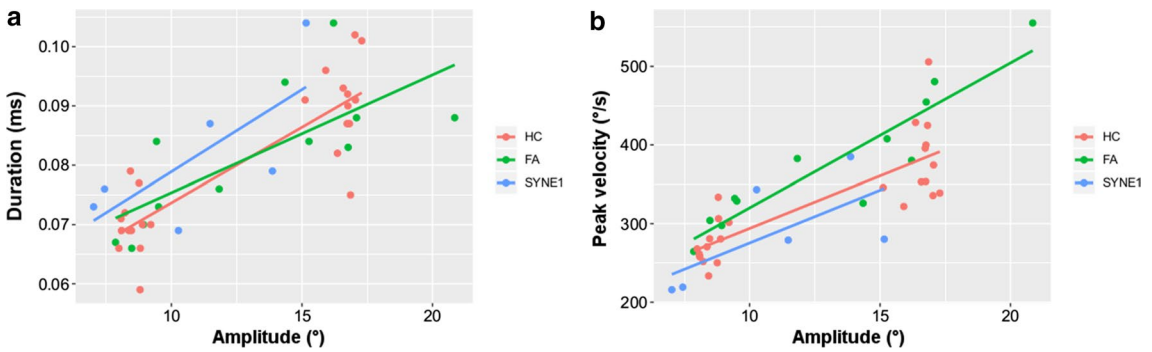

Fig. 4 The main sequence relationships of saccades using the linear model. **a**: saccadic duration versus amplitude; **b** saccadic peak velocity versus amplitude; the red, green and blue dots denote the parameters of healthy controls (HC), Friedreich ataxia patients (FA) and *SYNE1* subjects, respectively

Table 3 Antisaccade examination in *SYNE1* (AT-04–06) and Friedreich ataxia patients and in healthy controls

Subjects	9.2° antisaccades				18.4° antisaccades			
	Peak velocity (°/s)	Latency (s)	Duration (s)	Incorrect ratio	Peak velocity (°/s)	Latency (s)	Duration (s)	Incorrect ratio
AT-04	261.71	0.28	0.053	0.40	290.23	0.29	0.052	0.27
AT-05	232.76	0.32	0.072	0.64	280.16	0.37	0.097	0.36
AT-06	212.44	0.19	0.077	1.00	221.08	0.41	0.069	0.35
Median FA (range)	300.87 (237.22–351.78)	0.28 (0.19–0.41)	0.067 (0.059–0.072)	0.62 (0.28–0.96)	336.57 (253.63–439.47)	0.32 (0.20–0.45)	0.083 (0.062–0.103)	0.33 (0.06–1.00)
Median HC (range)	243.06 (197.67–313.18)	0.28 (0.23–0.33)	0.053 (0.046–0.075)	0.19 (0.08–0.54)	283.15 (209.63–382.10)	0.28 (0.24–0.37)	0.062 (0.049–0.088)	0.07 (0.00–0.29)

FA Friedreich ataxia, *HC* healthy control

Table 4 Neuropsychological assessment of *SYNE1* and Friedreich ataxia patients

Patient code	Age (years)	Education (years)	ACE (93.7 ± 4.3)	MMSE (28.8 ± 1.3)	LST	BDST	Verbal fluency	Semantic fluency	RCFT copying	RCFT recall
SYNE1 patients										
AT-04	35	14	89	29	2 (3.38 ± 0.79)[a]	4 (5.88 ± 1.1)[a]	10.5 (17.61 ± 5.42)[a]	14 (17.25 ± 3.96)	NA	NA
AT-05	43	14	93	29	2.33 (3.38 ± 0.79)[a]	3 (5.88 ± 1.1)[b]	14.5 (17.61 ± 5.42)	17 (17.25 ± 3.96)	35 (31.1 ± 3.6)	15 (23.7 ± 5.2)[a]
AT-06	37	12	89	29	2 (3.38 ± 0.79)[a]	2 (5.88 ± 1.1)[c]	16 (17.61 ± 5.42)	14 (17.25 ± 3.96)	36 (31.1 ± 3.6)	21.5 (23.7 ± 5.2)
Friedreich ataxia patients										
AT-08	23	12.5	93	30	3 (3.45 ± 0.89)	4 (5.88 ± 1.1)[a]	14.5 (16.13 ± 5.65)	11 (15.84 ± 4.51)	36 (31.1 ± 3.6)	24 (23.7 ± 5.2)
AT-11	57	16	87[a]	27	2.33 (3.11 ± 0.61)[a]	3 (5.34 ± 0.96)[b]	12 (11.02 ± 4.98)	16 (13.77 ± 4.05)	36 (29.2 ± 4.2)	26.5 (15.5 ± 5.5)
AT-12	60	17	95	29	3 (3.11 ± 0.61)	2 (5.34 ± 0.96)[c]	15.5 (11.02 ± 4.98)	20 (13.77 ± 4.05)	34 (29.2 ± 4.2)	27 (15.5 ± 5.5)
AT-20	16	10	88	26[a]	2.66 (3.33 ± 0.59)	5 (5.88 ± 0.96)	13.5 (13.83 ± 4.31)	14 (13.44 ± 3.52)	36 (31.1 ± 3.6)	26 (23.7 ± 5.2)
AT-21	59	14	94	30	3 (3.11 ± 0.61)	5 (5.34 ± 0.96)	15 (11.02 ± 4.98)	14 (13.77 ± 4.05)	32 (29.2 ± 4.2)	17.5 (15.5 ± 5.5)
AT-22	34	15	96	30	3.3 (3.38 ± 0.79)	4 (5.88 ± 1.1)[a]	16.5 (16.13 ± 5.65)	21 (15.84 ± 4.51)	34 (31.1 ± 3.6)	21 (23.7 ± 5.2)

In ACE and MMSE the lower normal threshold values of the normal population are in the brackets

In LST, BDST, verbal fluency, semantic fluency, RCFT copying and RCFT recall the age- and education-matched lower threshold values of the normal population are in the brackets

ACE Addenbrooke's Cognitive Examination, *BDST* Backward Digit Span Task, *LST* Listening Span Task, *MMSE* Mini-Mental State Examination, *NA* not available, *RCFT* Rey Complex Figure Test

[a] Mild deficit, [b]moderate deficit, [c]severe deficit (Age- and education-matched standards, and standard deviations of the literature are delineated [23–25]. Mild, moderate and severe deficits mean that the cognitive impairment of the subject is more pronounced than one, two and three standard deviations of the normal standards, respectively)

patients, however these observations were based exclusively on physical examinations [1–4, 18, 19]. Our findings, obtained by fine eye tracking assessment, confirmed the clinical observations of some earlier publications, i.e., a high frequency of slow saccades can be detected in *SYNE1* ataxia. This lower saccadic velocity is likely due to brainstem involvement, in particular, the functional loss of pontine saccadic burst generator neurons and omnipause neurons can explain this observation [23]. Slowing of saccades is a characteristic eye movement abnormality in SCA2 disease. Federighi et al. examined the saccadic parameters of seven SCA2 patients at similar target eccentricities (10 and 18°) to those we used in this study and they found more severely reduced peak velocities and delayed saccadic latencies compared to controls than we detected in two of three *SYNE1* patients [23]. Presumably brainstem impairment is more pronounced in SCA2 than in *SYNE1*. In addition, saccadic hypometria was not found in SCA2 patients, whereas we observed lower saccadic amplitude in *SYNE1* patients compared to healthy subjects at the larger stimulus paradigm.

The antisaccade assessment showed higher rates of incorrectly accomplished antisaccades in both FA and *SYNE1* patients compared to healthy subjects, whereas the other parameters were in similar ranges. The error rates were higher on the short stimulus amplitude task than on the long amplitude trial. Basically, target eccentricities affect gain, latency and peak velocity, whereas its influence on the incorrect ratios is not clear at these amplitudes [29–31]. The higher error rate raises the suspicion of cognitive impairment, because a strong correlation was demonstrated between antisaccades and working memory [32]. The neuropsychological assessment revealed that global cognitive performance was normal in *SYNE1* patients, whereas executive functions were impaired, especially working memory. The performance of the examined *SYNE1* subjects in BDST and LST paradigms inversely correlated with errors in the antisaccade tasks, i.e., the most severely affected patient in working memory tests (AT-06) had the highest error rate in the antisaccade paradigm (Fig. 5). A similar relationship was not detected in the FA group. Previously published studies indicated higher antisaccadic error rates in other hereditary and idiopathic cerebellar disorders, including ataxia with oculomotor apraxia type 1, 2, ataxia telangiectasia, SCA1, 2, 3 and late onset cerebellar ataxia (LOCA) [28, 33–35]. Additionally, Pretegiani et al. revealed that SCA2 and LOCA patients showed equally poor antisaccade performance irrespective of cortical involvement [33]. Additionally, a thorough investigation of a SCA2 patient cohort confirmed that impaired antisaccade efficacy was associated with executive test deficits, including Stroop interference task and verbal fluency test [34]. Our findings draw attention to the major role of working memory and inhibitory control in the performance of antisaccades, and confirm that executive dysfunction is a prevalent neuropsychological abnormality in hereditary ataxias as a part of the cerebellar cognitive and affective syndrome [36].

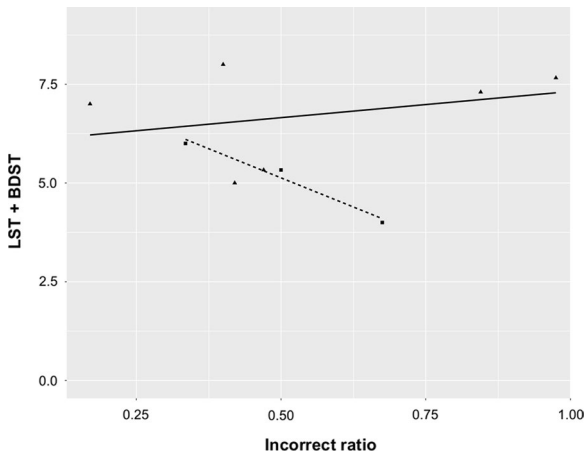

Fig. 5 The delineation of the possible relationship between working memory test results and the incorrect ratio of antisaccades in ataxia patients. The horizontal axis denotes the mean value of the incorrect ratios of 9.2 and 18.4 antisaccade tasks, whereas the vertical axis indicates the sum of Listening Span Task (LST) and Backward Digit Span Task (BDST) scores; the triangles and squares indicate the data of Friedreich ataxia patients (FA) and *SYNE1* patients, respectively, and the regression line is drawn by continuous and dashed lines

Conclusions

In conclusion, this paper demonstrates the detailed neurological assessment of the first Hungarian *SYNE1* ataxia patients with novel pathogenic mutations. The eye tracking investigation detected some interesting alterations regarding both saccades and antisaccades in these subjects, including saccadic hypometria and increased error rates for antisaccades. The main weakness of this study is the low case number. Nevertheless, these pilot findings point out the importance of device-aided examination of eye movements in ARCAs. Hopefully in the near future, these parameters can be investigated in a larger number of *SYNE1* patients in order to be able to draw statistical conclusions as well.

Abbreviations

ACE: Addenbrooke's Cognitive Examination (ACE); ARCA: Autosomal recessive cerebellar ataxias; ARCA1: Autosomal recessive cerebellar ataxia type 1; BDST: Backward Digit Span Task; FA: Friedreich ataxia; HC: Healthy controls; LOCA: Late onset cerebellar ataxia; LST: Listening Span Task; MMSE: Mini-Mental State Examination; NGS: New generation sequencing; RCFT: Rey Complex Figure Test; SARA: Scale for the Assessment and Rating of Ataxia; SCA: Spinocerebellar

ataxia; SCAR8: Spinocerebellar ataxia, autosomal recessive 8; WES: Whole exome sequencing.

Acknowledgements
The linguistic corrections were made by a native English reader, Jennifer Tusz from Canada.

Authors' contributions
LSZ examined the patients and wrote the manuscript. GSZ and BK performed the eye-tracking examination on the subjects. BK set up the eye-tracking aided device and established the methods of the study. VLN and NSZ performed the neuropsychological examination of the patients. ZM, TK, MR and RP carried out the new generation sequencing of the *SYNE1* patients. GV analysed the data and made the diagrams. PK and AS had important recommendations to the manuscript. DZ was the major contributor in writing the manuscript. All authors read and approved the final manuscript.

Funding
This work was supported by KTIA Grant No. 13 NAP-A-II/17. Open access funding provided by the University of Szeged (SZTE, Grant Number: 4906).

Availability of data and materials
The datasets used and/or analysed in the current study are available in this paper.

Ethics approval and consent to participate
Written informed consent was obtained from the patients for the publication of this study (Regional Human Biomedical Research Ethics Committee of the University of Szeged, registration number 44/2016). All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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Received: 17 September 2020 Accepted: 13 January 2021
Published online: 01 February 2021

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VII.

BMC Neuroscience

CORRECTION

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Correction to: Eye-tracking-aided characterization of saccades and antisaccades in SYNE1 ataxia patients: a pilot study

Laszlo Szpisjak[1], Gabor Szaraz[1], Andras Salamon[1], Viola L. Nemeth[1], Noemi Szepfalusi[1], Gabor Veres[1,5], Balint Kincses[2], Zoltan Maroti[3], Tibor Kalmar[3], Malgorzata Rydzanicz[4], Rafal Ploski[4], Peter Klivenyi[1] and Denes Zadori[1*]

Correction to: BMC Neurosci (2021) 22:7 https://doi.org/10.1186/s12868-021-00612-9

Following publication of the original article [1], the authors reported an error in Fig. 2b. The description of the mutation in the Intron 128–Exon 128 boundary is inappropriate as using the terminology for codons is restricted only for exons, and it cannot be applied at this site. Furthermore, the number of the intron preceding exon 128 should be marked as 127. Regarding the identified error the text itself needs the following minor correction in the second paragraph in page 7 of 12: 'It causes a TAG–TGG codon change at the Intron 128–Exon 128 boundary resulting in an abnormal splicing variant (Fig. 2b).' to the following 'It causes an A>G change at the Intron 127–Exon 128 boundary resulting in an abnormal splicing variant (Fig. 2b).'

The correct Fig. 2 is included in this Correction article, and the original article has been updated.

The original article can be found online at https://doi.org/10.1186/s12868-021-00612-9.

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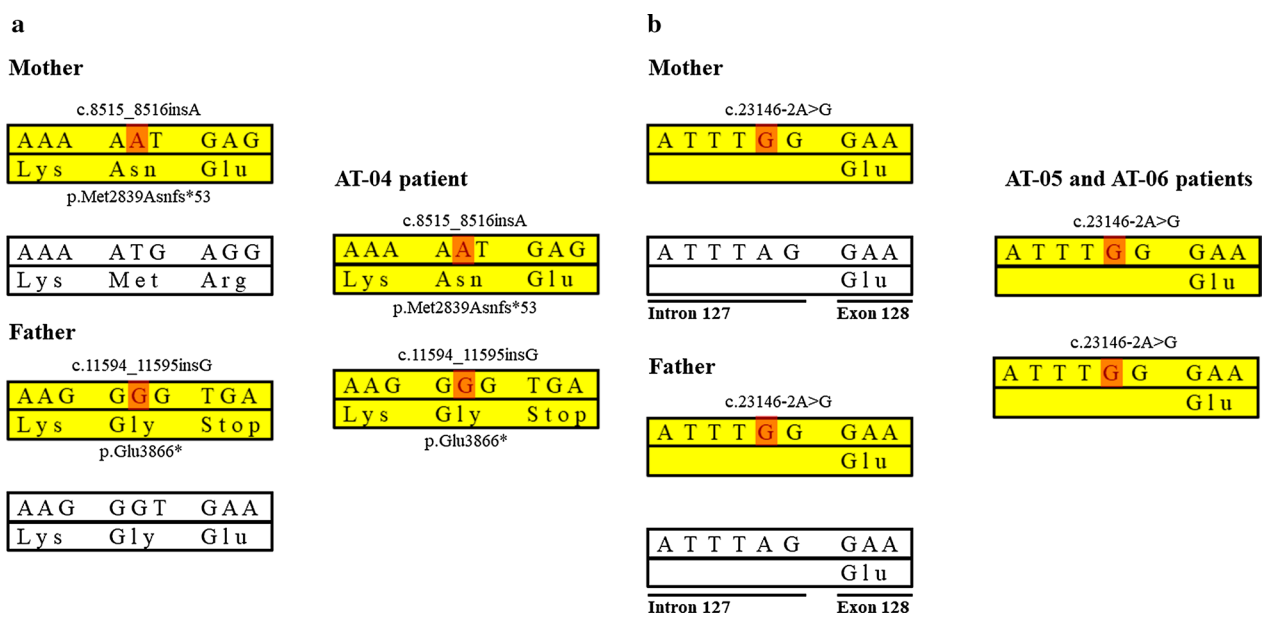

Fig. 2 Genetic abnormalities and consequent alterations of protein of *SYNE1* ataxia patients and their parents. **a** *SYNE1* gene mutations in AT-04 patient and the parental origin of these variations. **b** *SYNE1* gene abnormalities in AT-05 and AT-06 subjects and the parental segregation of these mutations. The upper parts of the bars denote the DNA sequence, while the lower parts show the encoded amino acids of the protein. Yellow bars indicate the pathogenic alleles, white bands mark the normal alleles. Red highlights the nucleotide change of the *SYNE1* gene. In part **b**, the c.23146-2A>G mutation is located in the intron–exon boundary resulting in an abnormal splicing variant

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Published online: 10 March 2021

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