# CLINICAL AND GENETIC CHARACTERIZATION OF HEREDITARY ATAXIAS

PH.D. THESIS

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# 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 limb and gait ataxia and other cerebellar symptoms. Besides the motor signs, cerebellar lesions can cause cognitive and affective disturbances as well, involving the impairment of executive functions, visuo-spatial organization and memory, language skill deficits and affective changes. This clinical observation was the basis of the entity of cerebellar cognitive affective syndrome (CCAS) or Schmahmann syndrome. In ataxic disorders the most important aspects of the medical history are the age at onset of ataxia, 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, which are characterized by acute or subacute onset and can be originated from vascular, autoimmune, infectious, toxic, malignant, vitamin deficiency or endocrine causes. Therefore, the initial work-up including brain magnetic resonance imaging (MRI), routine and special laboratory investigations, cerebrospinal fluid examinations and a detailed anamnestic survey of cerebellar symptoms. Following the exclusion of secondary causes, hereditary and non-hereditary degenerative disorders should be considered as the cause of CA, where the disease course is usually slowly progressive. Regarding the age at onset, early-onset CA is defined as symptoms beginning before the age of 25 years, while in adult-onset CA the complaints appear after 40 years. Sporadic, non-hereditary CAs are adult-onset disorders and the most common disease of this subgroup is multiple system atrophy cerebellar type (MSA-C) with the core clinical feature of autonomic dysfunction.

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. Autosomal dominant CAs (ADCAs), also called as spinocerebellar ataxias (SCAs), are a heterogenous group of hereditary disorders characterized by the cerebellar symptoms as their main clinical features. Currently, more than 40 genetically distinct subtypes of SCA are known. 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. PolyQ SCAs include

SCA1, 2, 3, 6, 7, 17 and dentatorubro-pallidoluysian atrophy (DRPLA). Besides the polyQ group, disease-causing repeat expansions have been identified in non-coding regions in some SCAs and conventional mutations can cause certain types of SCA, as well. Autosomal recessive CAs (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 late-onset as well. 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. The most common disease in this group is Friedreich's ataxia (FA), which is caused by biallelic pathogenic variants in the *FXN* gene. 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.

SCA28 was described in 2006 as a juvenile or young adult onset, slowly progressive ataxia with eye movement abnormalities. The causative gene, *AFG3L2*, encodes the mitochondrial AFG3L2 protein, which together with paraplegin is a subunit of m-AAA protease complex and has a major role in the quality control of mitochondrial proteins. The most frequent clinical characteristics of SCA28 patients are gait and limb ataxia, dysarthria, nystagmus, ophthalmoparesis, ptosis, slow saccades and pyramidal symptoms.

Ataxia with oculomotor apraxia type 2 (AOA2) is an early onset ARCA caused by mutations in the *SETX* gene. The onset of the disorder is typically between 12-20 years of age and the most common clinical features are cerebellar symptoms, sensorimotor polyneuropathy (PNP), oculomotor apraxia (OMA), pyramidal signs and involuntary movements such as head tremor, dystonia and chorea. The most important laboratory biomarker is elevated serum alpha-fetoprotein (AFP) and occasionally higher serum creatin-kinase (CK) levels. Brain MRI shows severe cerebellar atrophy. *SETX* gene encodes the DNA/RNA helicase protein senataxin, which has a significant role in genome stability, degradation and stress granule disassembly.

*AARS2*-associated leukoencephalopathy is a rare type of early or young adult-onset leukodystrophy (LD) characterized by cerebellar symptoms, cognitive deterioration, psychiatric abnormalities, pyramidal signs and occasional epilepsy and dystonia with ovarian failure in females. The typical brain MRI alterations of the disease are confluent, asymmetric

white matter abnormalities sparing the U-fibres, predominantly in the frontoparietal and periventricular region, involvolving the corpus callosum, pyramidal and other descending tracts, and cerebellar atrophy. The nuclear gene *AARS2* encodes the mitochondrial alanyl-tRNA synthase protein, which is involved in mitochondrial translation. *AARS2* was first identified as the causative gene of fatal, early-onset cardiomyopathy in 2011.

Cerebrotendinous xanthomatosis (CTX) is an AR disease of metabolism with the main characteristic neurological symptoms of cerebellar ataxia, pyramidal symptoms, cognitive decline, parkinsonism and seizures. The most prevalent extraneurological abnormalities are diarrhea, juvenile cataract, tendon xanthomas and psychiatric disturbances. The causative gene is the *CYP27A1*, encoding the sterol 27-hydroxylase protein, which takes part in the appropriate production of bile acids from cholesterol, whereas its deficiency results in the accumulation of undesired lipid metabolites. Specific laboratory finding of CTX is an 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.

Xeroderma pigmentosum (XP) is a rare AR condition with geographically variable prevalence. There are several subtypes of XP marked with letters A-G and the variant form based on the different genetic background. Besides the characteristic cutaneous and ophthalmological abnormalities, the most frequent neurological symptoms involve cerebellar ataxia, cognitive decline, speech disturbance, sensorineural hearing loss, PNP and pyramidal signs. The neurological signs occur almost in all patients of the XPA subtype. The typical brain MRI disturbances of XPA subjects demonstrated age-dependent abnormalities, including severe, diffuse brain atrophy, decreased fractional anisotropy value in diffusion tensor imaging and reduced N-acetyl aspartate/creatine ratio in MR spectroscopy.

Autosomal recessive cerebellar ataxia type 1 (ARCA1) is a rare neurodegenerative disorder caused by biallelic mutations of the *SYNE1* gene. The gene encodes the huge 8797 amino acids containing peptide Nesprin 1, which is a member of the spectrin protein family and its major function is linking the cell membrane to the actin cytoskeleton. *SYNE1* ataxia was described first 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. 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. The most frequent extracerebellar neurological signs are upper and lower motoneuron symptoms whereas non-neurological abnormalities include scoliosis, pes cavus and occasionally, respiratory dysfunction with severe manifestation.

# 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 by the 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, followed by making a pedigree, 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. 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, inclusive of serum levels of vitamin E, CK, albumin, lipids, lactate, AFP, iron, ferritin, immunoglobulins, ceruloplasmin, and rarely,

cholestanol. For purposes similar to laboratory examinations, brain MRI, and occasionally, other neuroimaging modalities were assessed to differentiate between the possible causes of CA. Moreover, functional neurophysiological methods were utilized. 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.

### 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, the most common repeat expansion hereditary ataxias (SCA 1, 2, 3, 6, 7 and FA) were investigated. 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. In most of our cases, the type of NGS was clinical exome sequencing (CES), 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. In addition to CES, for one patient, whole exome sequencing (WES) was performed.

### III/3**.** NEUROPSYCHOLOGICAL ASSESSMENT

A detailed neuropsychological assessment was performed in the following types of hereditary CA patients: SCA28, XPA and *SYNE1* ataxia by trained neuropsychologists. 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, Addenbrooke's Cognitive Examination (ACE) incorporating the Mini-Mental State Examination (MMSE) was performed. Phonological immediate memory was measured with the Digit Span Task (DST). Visuospatial immediate memory was assessed with the Corsi Block-Tapping Test (CBTT) and the Brief Visuospatial Memory Test-Revised (BVMT-R). Working memory was measured with the BDST (Backward DST) and the LST (Listening Span

Task). Letter, verb, episodic, and semantic fluency tests and the Wisconsin Card Sorting Test (WCST) were performed as well to assess executive functions. Everyday memory functions, including semantic memory, were measured with subtests of the Rivermead Behavioural Memory Test (RBMT). Visual attention and task switching functions were investigated with the Trail Making Test (TMT). 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. In case of *SYNE1* ataxia subjects, the following tests were assessed: ACE, MMSE, verbal and semantic fluency examination, BDST, LST. the quality of information planning and visuo-constructional and visual organizational abilities were assessed by the Rey Complex Figure Test (RCFT).

### III/4. EYE-TRACKING

Three *SYNE1* ataxia, 6 FA patients and 12 healthy controls (HC) were enrolled in the study. The assessment was performed with Tobii TX300 eye tracker and tasks were programmed in Psychophysics Toolbox V 3.0.12, under MatLab. 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. In the antisaccade task, the simple antisaccade paradigm was used. 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). 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. 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. 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 signs of ataxia and impaired coordination. Her symptoms appeared 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. Her parents and her brother did not report any neurological problems. The serum AFP level was elevated, whereas the brain MRI demonstrated moderate cerebellar atrophy with normal supratentorial structures. Genetic investigation for FA resulted in normal GAA repeat numbers. 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, 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. The allele frequency of the c.502C>T missense variant is 2/250,950 according to the Genome Aggregation Database (gnomAD) and is predicted to be deleterious by SIFT, and probably damaging by PolyPhen2 softwares. We presume that the compound heterozygous state of these mutations is responsible for the *SETX* insufficiency and the AOA2 disease.

## IV/2. *AARS2*-associated leukoencephalopathy study

The patient was a 29-year-old male with normal perinatal period and childhood. The behavioral changes of the subject began at the age of 18 years with the alteration of personality, mania and paranoid delusions. His intellectual development was normal with an excellent performance in schools until the age of 24 years, when his cognitive impairment became obvious and a rapid and progressive intellectual decline was started. During the following 2 years he developed

acalculia, orientation problems and dysgraphia. Moreover, the neurological examination revealed cerebellar symptoms, dysarthria, dysphagia, parkinsonism, pyramidal and frontal liberation signs as well. 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 LD, with predominant white matter abnormalities in the frontal and parietal lobes, with a relative sparing of the central region, involvement of the pyramidal tract, and moderate atrophy of the cerebellum and corpus callosum. Detailed laboratory examinations were performed to determine the etiology of this LD, but these tests did not demonstrate any pathological abnormalities. Targeted genetic tests for the *NOTCH3* gene and the most common vanishing white matter disease causing gene *EIF2B5* were also negative. After the reevaluation of the clinical phenotype and MRI features, a new entity, the *AARS2*-associated leukoencephalopathy seemed to be the most likely diagnosis. Targeted gene sequencing of *AARS2* gene was performed and it identified two possibly pathogenic mutations: c.578T>G and c.595C>T. The c.578T>G mutation was a new variant causing a nonsense mutation (p.Leu193*) in exon 3 and is likely to result in messenger RNA degradation by nonsense-mediated decay. 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. Segregation analysis proved the compound heterozygosity of the pathogenic mutations. Before the identification of the genetic abnormality, a biopsy sampling from the frontal lobe, including cortex and white matter, was performed by another department. Nevertheless, there was a lack of any specific pathological hallmark in this specimen.

### IV/3. CTX study

The proband was a 40-year-old female patient from a twin pair with movement disorder, childhood onset cataracts and glaucoma. Her movement and speech only started to deteriorate progressively three years before her admission. Her parents mentioned memory disturbances, anxiety, impatience and an episode of pronounced diarrhea. The neurological check-up revealed parkinsonism, mild cerebellar ataxia and pyramidal signs. The neuropsychological assessment demonstrated moderate cognitive impairment in light of 65/100 points in ACE and 24/30 points in the MMSE. The brain MRI showed T2 and FLAIR signal abnormalities in the dentate nuclei and some supratentorial white matter alterations. The clinical features and the MRI disturbances raised the possibility of CTX, therefore serum cholestanol measurement was performed, which

was elevated as well. The targeted genetic testing of the *CYP27A1* gene resulted in 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, however, she did not complain any neurological problems. The physical examination revealed gentle sensory ataxia with a slightly broad-based gait and signs of discrete parkinsonism. The neuropsychological assessment detected mild cognitive impairment. The brain MRI showed very similar signal abnormalities in the dentate nuclei, whereas elevated serum cholestanol levels were found as well. The genetic testing identified the same disease-causing mutation in the *CYP27A1* gene. The analysis of 15 short tandem repeat markers confirmed that the sisters are identical twins, whereas the segregation analysis demonstrated that their parents were heterozygous for the assessed mutation.

### IV/4. XPA study

The 36-year-old Caucasian male proband 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 with slurred speech and cognitive dysfunction. In the course of his disease, repeated falls, swallowing difficulties and visual disturbances developed as well. Upon neurological examination, eye movement disturbances, dysarthria, dysphagia, hypo-/areflexia, pathological reflexes, decreased sense of vibration and movement disorder with dominating ataxia and parkinsonism were noticeable. In addition to the neurological problems, slightly exaggerated sunburn reaction was observed on the skin. 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. The electroneurography (ENG) revealed mixed type sensorimotor PNP with lower limb predominance. He died at the age of 39 from aspiration pneumonia. Following his death, a comprehensive post mortem neuropathological examination was performed, which showed prominent generalized brain atrophy. The most prominent alterations of the microscopic neuropathological examination were asymmetrical hippocampal sclerosis, Purkinje cell degeneration along with moderate loss of neurons in the substantia nigra and a scattered infiltration of CD8-positive T lymphocytes. 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 and his three brothers. The main causes of dominantly inherited ataxia (SCA1-3 and 17) and cognitive dysfunction (*FMR1*, *PSEN1*, *PSEN2* and *APP* genes) were assessed first, finding no relevant alteration. Therefore, CES was performed, which identified two novel deletions of the *XPA* gene in exon 4 and 6. Based on the American College of Medical Genetics and Genomics variant interpretation guidelines, the exon 4 mutation was classified as likely pathogenic, whereas the exon 6 deletion as pathogenic. The segregation analysis proved the compound heterozygosity of the patient. Both of these mutations were identified in all siblings of the proband as well.

### IV/5. SCA28 study

Five affected patients from a Hungarian family were referred to our clinic with the suspicion of hereditary ataxia whose family tree suggested an AD inheritance pattern. The first complaint of the proband appeared at the age of 15 years as clumsiness of the limbs, later he developed speech disturbance, uncoordinated gait and mild double vision. The neurological assessment of the family revealed cerebellar symptoms of varying severity with dysarthria and eye movement abnormalities. Routine laboratory parameters were in the normal range, except mild elevation of serum total cholesterol and CK levels in some cases. The brain MRI revealed mild to moderate cerebellar atrophy predominantly in the vermis in 4 out of 5 patients. According to AD inheritance, the most common polyQ SCAs were tested first, however, the CAG repeats of SCA1, 2, 3, 6, and 7 were in the normal range. In the next step, CES was performed for the proband, which 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 subject of the family. In the position of this variant, two other pathogenic mutations were detected earlier and the identified amino acid change is located within a highly conserved region of the protein AFG3L2. The neurocognitive investigation demonstrated slight disturbances in complex working memory, visuospatial memory, semantic memory and executive functions.

### IV/6. *SYNE1* ataxia study

The AT-04 subject 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. He had strabism and myopia with negative fundoscopy. ENG demonstrated mild axonal sensory PNP. 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. The repeat expansion tests of FA and the most common polyQ SCAs were negative. WES identified two frameshift mutations in *SYNE1* gene, whereas the segregation analysis proved the compound heterozygous state of these variants. None of the frameshift mutations 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) of gait problems were referred to our clinic. Their first symptom was gait ataxia at the age 30 years (AT-05) and 14 years (AT-06). The neurological examination of both patients revealed cerebellar dysarthria, brisk tendon reflexes with bilateral Babinski signs and ataxia of the limbs and trunk. Both patients had obesity, diabetes mellitus, hypertension and hypercholesterolemia, whereas AT-06 subject had excavated foot and multifocal sensorimotor mixed type PNP as well. The brain MRI revealed moderate cerebellar and very mild cerebral cortical atrophy in both patients. Their non-consanguineous parents did not suffer from ataxia. In AT-05 and AT-06 patients the same homozygous c.23146-2A > G intronic variant of the *SYNE1* gene was detected, which was not found in gnomAD. This mutation causes an abnormal splicing variant in Intron 127–Exon 128 boundary. Segregation analysis identified this variant in the heterozygous state in both parents of the subjects. The eye-tracking examination did not find 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. The main sequence relationships demonstrate that saccades of *SYNE1* ataxia patients are hypometric, their duration is longer and their peak velocity is lower than in FA or HC groups. In the antisaccade paradigm, there was no remarkable difference between the groups with regard to peak velocities, latencies and durations. The incorrect ratios were higher in the *SYNE1* and FA patients than in the HC group. In the neuropsychological assessment, 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 that of the ability to maintain and manipulate information.

# 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 and 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). The milder phenotype of our patients suggests a residual activity of senataxin.

In the *AARS2*-associated leukoencephalopathy study we described a young male patient with LD caused by compound heterozygous state of a known pathogenic missense (c.595C>T) and a novel nonsense (c.578T>G) variants 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. 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. 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.

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. 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. 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, the deletion of which results in ineffective binding of XPA protein to replication protein A. The exon 6 frame-shift mutation may be considered pathogenic as it leads to a premature stop codon. 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 a manifest skin lesion. 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. One possible explanation of this difference, that these signs appear later in the disease, whereas only two subjects were older than 50 years amongst our patients. In addition to the genetic finding and the comparison of clinical features, detailed neurocognitive assessment were 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 the most common polyQ SCAs and might be the part of the CCAS.

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 PNP was observed as well. The clinical features of our patients were not purely cerebellar, similarly the European ARCA1 subjects published by Synofzik and Mademan et al. This heterogeneity of symptoms and the other *SYNE1* associated diseases suggests that *SYNE1* plays a significant role in the proper functioning of the nervous and musculoskeletal systems as well. Despite the numerous symptoms of the discovered hereditary diseases, an obvious genotype-phenotype correlation has not been established so far. The eye-tracking assessment demonstrated hypometric saccades in all *SYNE1* ataxia patients in the longer paradigm and in two out of three in the shorter task. Saccadic dysmetria is a common, but not specific cerebellar symptom in the disease group of hereditary ataxias, however, there may be a higher frequency of hypo- or hypermetric saccades, serving as a supporting clinical feature of the disease. 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. In addition to accuracy, velocity is a relevant feature of saccades as well, whereas the evaluation of it is often difficult by physical examination. The importance of current research is the confirmation of 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 neuron and omnipause neurons. Between hereditary CAs, the low saccadic velocity was a characteristic feature of SCA2,

however, at similar conditions, 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.

The antisaccade examination demonstrated higher rates of incorrect antisaccades in both FA and *SYNE1* ataxia patients compared to HC. 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. This correlation was proven by neuropsychological assessment, which showed executive dysfunction with particular involvement of the working memory, whereas the values scored by *SYNE1* subjects in BDST and LST tests demonstrated inverse correlation with the error rate of antisaccade paradigm. 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. 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.

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(For references to the statements in the thesis booklet, see the dissertation.)

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

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