Neurocognitive characterization of an SCA28 family caused by a novel *AFG3L2* gene mutation

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Abstract

Spinocerebellar ataxia 28 (SCA28) is an extremely rare, autosomal, dominantly inherited, juvenile onset, slowly progressive, gait and limb ataxia with frequent eye movement abnormalities and cerebellar atrophy. The causative gene of SCA28 is AFG3*L2*, located on the short arm of chromosome 18. In this paper we demonstrate the neurocognitive assessment of 5 affected patients in the first Hungarian SCA28 family. The identified c.2011G>C heterozygous base pair change is a novel point mutation variation resulting in an already known p.Gly671Arg amino acid change. The previously described 82 SCA28 patients were compared with our patients and we found that the majority of clinical features, including early onset, slow progression, gait and limb ataxia, dysarthria and pyramidal symptoms are similar to the alterations characteristic of other SCA28 patients. Some ophthalmological manifestations, such as ptosis, ophthalmoparesis and slowing of saccades are not present in our patients. Since detailed psychological investigation was not performed previously in SCA28 patients, the following major neuropsychological functions were examined: phonological immediate memory, visuospatial immediate memory, working memory, executive functions, everyday memory functions including semantic memory, visual attention and speed of processing. The results of these assessments demonstrated slightly lower levels of performance in complex working memory, visuospatial memory, semantic memory and executive functions with some variation between subjects. These abnormalities may be the consequence of alterations in the cerebellar-prefrontal connection system.

Keywords

SCA28, AFG3L2, autosomal dominant cerebellar ataxia, cognition, spinocerebellar ataxia

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].

SCA 28 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. The family tree was also delineated (Figure 1). Routine laboratory examination was carried out on all the affected subjects, while brain MRI was performed on 4 out of 5 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 4 out of 5 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; 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, the 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 2 of out of 5 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 (Figure 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 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 (Figure 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, 7 were in the normal range, so in the next step clinical exome sequencing was performed for Patient 1 (IV-5; Figure 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, dbSNP) from which only 3 have autosomal dominant inheritance. We excluded *ITPR1* NM_001168272.1:c.1435G>A (rs41289628) and *CACNA1A* NM_023035.2:c.6976 6993delCAGCAGCAGCAGCAGCAGinsCAGCAGCAGCAGCAG 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 (Figure 3/a). This novel mutation was not found in either the 148 unrelated Hungarian controls, nor 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; Figure 1 and Figure 3), but was not found in a healthy sister (V-4) of Patient 3 (Figure 3/a). The variant is located in exon 16 of the gene (Figure 3/c). 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 (Figure 3/b).

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 the disease. In our family, only Patient 1 and 2 are older than 50

years, while the mean age of these ophthalmological signs of the previously reported SCA28 patients were 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, visuospatial immediate memory and semantic memory, and in executive functions. These abnormalities are similar to the cognitive alterations 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 [33, 34] 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 [35]. This report described 20 patients (13 cerebellar stroke, 3 postinfectious cerebellitis, 3 cerebellar cortical atrophy and one excision of a midline tumor) with CCAS [35]. Malm et al. also reported young adult patients (aged 18 to 44 years) with infratentorial infarcts having symptoms of the CCAS in 1998 [36]. Neau et al. described the same cognitive disturbances in elder patients (aged: 39-75 years) with cerebellar infarcts in 2000 [37]. Fitzpatrick et al. investigated patients (mean age: 53.41 years) with alcoholic cerebellar degeneration and delineated similar cognitive abnormalities in 2013 [38]. 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 [39, 40]. 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 [41]. 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, posterior vermis and the fastigial nucleus. In this context, our patients demonstrated only mild deficits in executive and visuospatial functions. We 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, but the presentation of a single family may conversely decrease this variability by avoiding some interfamiliar 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 had 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 [42]. These pilot findings may enhance the setup of better genotype-phenotype correlations in SCA 28 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.

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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.

Tables (with captions)

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 [30]	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.

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

Mi: mild, Mo: moderate, S: severe, UL: upper limbs, LL: lower limbs. As the available data are limited from the literature, the numbers of affected individuals are presented out of the reported patients.

Figures (with captions)

Figure 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.

Figure 2. Brain MRIs of Patient 1 and 3. Sagittal, FLAIR-weighted (a) and axial, T2-weighted image (b) of Patient 1 demonstrates mild cerebellar atrophy, mainly in the vermis. Sagittal, T2-weighted (c) and axial FLAIR-weighted picture (d) of Patient 3 indicates moderate cerebellar atrophy.

Figure 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.