Examination of the features of trinucleotide expansion associated polyglutamine diseases in Hungarian and international patient groups

PhD thesis

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ABBREVIATIONS

8MW: 8m walk test

9HPT: 9-hole peg test

FRDA: Friedreich's ataxia

MSFC: Multiple Sclerosis Functional Composite

PATA: The test rate refers to how often the proband can repeat the syllables „PATA" within ten seconds

SARA: Scale for the Assessment and Rating of Ataxia

SCA: spinocerebellar ataxia

SCAFI: SCA Composite Functional Index

SD: standard deviation

UHDRS-IV: Unified Huntington's Disease Rating Scale functional assessment, part IV

ICARS: International Cooperative Ataxia Rating Scale

INAS: Inventory of Non-Ataxia Symptoms

HD: Huntington's disease (Huntington chorea)

ADCA: Autosomal dominant cerebellar ataxia

DRPLA: Dentato-rubral-pallidoluysian atrophia

1. INTRODUCTION

The research of trinucleotide repeat disorders has been the most dynamically developing field of neurogenetics since the 1990s, in the course of which the molecular genetic background of numerous different clinical pictures has been explored. In the first stage of the work, the clinical characteristics of Huntington chorea were examined, and its frequently mentioned but rarely examined feature, the occurrence of suicidal behaviour among patients.

In the frame of international cooperation, from 2004 and 2008, we took part in the EUROSCA project funded by the Sixth Framework Programme of the EU; a consortium organised by twenty-two working groups in nine countries.

The primary aim of the clinical branch was the diagnosis and research of spinocerebellar ataxias (SCA), and, depending on the advances made, the drafting of possible principles and specific proposals.

The most common trinucleotide repeat mutation is the CAG repeat expansion which is coding the glutamine amino acid. The mutation causes an increased number of CAG repeat residues which leads to the expansion of a polyglutamin tract.

These disorders include the Huntington's disease (HD) and most of the spinocerebellar ataxias.

1.1 Huntington's disease

The disease was first described by George Huntington in 1872. Onset typically occurs between 30 and 40 years of age. It is characterised by progressive motor impairment (chorea), cognitive decline and mental disorders.

HD shows a prevalence of 3-10 affected individuals per 100,000. Neuropathological changes in Huntington's disease are prominent cell loss and atrophy in the caudate and putamen, but globus pallidum and cortical layers can also be affected.

One of the pathological characteristics of HD is the appearance of nuclear and cytoplasmatic inclusions that contain mutant huntingtin.

1.2 Spinocerebellar ataxias

The SCA-s form a genetically heterogeneous group of autosomal dominantly inherited progressive ataxia disorders. Up to now, more than 25 different genes or loci have been found. The prevalence is given as 3/100,000. In the known populations the SCA3 subtype is the most frequent worldwide. Up to now more then 24 different autosomal dominant ataxias as SCA1-8, 10-19, 21-23, 25 and 28 and DRPLA and FGF14 ataxias are known. Six SCA types (SCA1, 2, 3, 6, 7 and 17) and DRPLA are caused by pathologic CAG repeat number expansions in different genes. Most of the genes that cause the diseases were found to encode proteins of unknown functions. The SCA-s show a large degree of clinical heterogeneity, which makes clinical diagnosis often difficult.

2. AIMS OF THE STUDY

1. Study of demographic and psychiatrical characteristics of HD-patients, especially suicide.
2. To identify genetic modifiers influencing the age at disease onset, we searched for polymorphic markers in UCHL1 (S18Y) and the GRIK2, TBP, BDNF, HIP1 and ZDHHC17 genes.
3. The study evaluated clinical reliability and validity of the ICARS in SCA patients. In addition, functional disability using the Barthel Index was determined as external criteria.
4. Developing of a new clinical scale, the Scale for the Assessment and Rating of Ataxia (SARA) to measure the severity of ataxia.
5. We studied the nonataxia symptoms to identify factors that determine disease severity and clinical phenotype.
6. SCA Functional Index: Performing of new compound performance measure.

3. PATIENTS AND METHODS

3.1 Suicide in Huntington's disease patients

The families and patients were identified through their physicians, neurologists and psychiatrists. Most of them were living in the counties of Baranya, Tolna, Somogy, Zala, Veszprém, Győr-Moson-Sopron and Vas. Patients were found by clinical examination, medical records, death certificates and reports from relatives. Deaths between 1920 and 1997 were included.

3.2 Genetic analysis of modifier genes

After giving informed consent, blood was taken from 98 patients. Repeat numbers were analysed in the Department of Genetics in Pécs and Tübingen. The allele polymorphisms were screened in Tübingen.

3.3 S18Y polymorphism in the UCHL1 gene

The DNA samples of 946 unrelated HD patients were studied. The effect of the S18Y polymorphism together with the expanded CAG repeat on the HD age-at onset was analysed.

3.4 International Cooperative Ataxia Rating Scale ICARS

There is no effective therapy for SCA patients. To evaluate the efficacy of potential new therapies a former clinical scale was developed, the International Cooperative Ataxia Rating Scale (ICARS).

We evaluated the metric properties of the scale in 156 SCA patients and 8 healthy controls in 13 European centres.

3.5 Scale for the Assessment and Rating of Ataxia (SARA)

In its final form, SARA has eight items, that yield a total score of 0 (no ataxia) to 40 (most severe ataxia). Symptoms are: gait (score 0 to 8), stance (score 0 to 6), sitting (score 0 to 4), speech disturbance (score 0 to 6), finger chase (score 0 to 4), nose-finger test (0 to 4), fast alternating hand movements (score 0 to 4), heel-shin side (score 0 to 4). Limb kinetic movements were rated indepently for both sides and the arithmetic mean of both sides is included for the SARA score.

The study population consisted of 167 patients in Trial 1 and 119 in Trial 2. In the first Trial SARA was compared with disease stages, ICARS score, Barthel Index and in Trial 2 with disease stages, Barthel Index and UHDRS–IV. In addition, the severity of ataxia of 11 cases presented on video was globally assessed by examiners on a visual analogue scale ranging from 0 (no ataxia) to 100 (most severe ataxia).

3.6 Non-ataxia symptoms

To identify factors that determine disease severity and clinical phenotypes of the most common SCA-s a total of 526 patients with SCA1, SCA2, SCA3 or SCA6 were studied. The non-ataxia symptoms were assessed with the INAS. It consists of 16 symptoms in 30 items. DNA sample was available from 450 of the patients. In 53 participants, information about repeat lengths were taken from medical records, in 23 patients the repeat lengths were lacking. The SARA score was used to determine the severity of ataxia.

3.7 SCA Composite Functional Index SCAFI

The functional composite was performed in a subset of 412 patients in 15 European centres. The clinical assessment included the SARA score, UHDRS-IV, 9HPT, 8MW and PATA tests. Information on cognitive disturbance was derived from rater-based assessment as mild, moderate or severe. All tests were repeated resulting in two values for each tests. The results of each subtest (9HPT, PATA and 8MW) were transformed into Z-scores expressed in SD from baseline mean. The Z-score is defined as follows: Z-score =(individuals average of both trials-mean of study population)/SD of study population. A functional index was generated as the arithmetic mean of all three Z-scores. Principally, higher Z-scores are equivalent to better performance in all three subtests.

4. RESULTS

4.1 Suicide in Huntington's disease patients

There were 396 deaths reported (195 males and 201 females) among those suffering from HD in 96 families in the period from 1920 to 1997. Forty suicides occurred in 27 families. Suicide was more common in the larger families. Suicide among close relatives was found in 4 of the families with 9 HD cases.

Of the 40 subjects who committed suicide, 34 were male and 6 were female; this was not significantly different from the expected number based on suicide rates in the general Hungarian population. In 98 of the 396 reported HD patient deaths, the patients died in hospital or psychiatric homes.

The age at onset of HD in the 396 HD patients who died was 40.4 years, and for the suicide patients, it was 37.8 years (not a statistically significant difference).

The suicide methods were different from those of the population at large. In about 70% of the general Hungarian population, suicide is by hanging, while suicide in HD patients was most frequently committed by drowning. In men, there was a high frequency of suicide by firearm shooting or by jumping in front of a vehicle. Self-poisoning was usually by chemicals and not drugs as in the general Hungarian population. (**Table 1**)

Table 1

Methods of suicide by HD patients

	Male	Female	Total (%)
Drowning	9	2	11 (27)
Hanging	7	2	9 (23)
Self-poisoning	8	1	9 (23)
Drug	2	1	
Gas	2	—	
Chemicals	4	—	
Jumping out of windows	4	1	5 (12)
Jumping in front of vehicle	4	—	4 (10)
Shooting	2	—	2 (5)
Total (%)	34 (85)	6 (15)	40 (100)

4.2 Genetic analysis of modifier genes

None of the genes encoding GRIK2, TBP, BDNF and ZDHHC17 could be identified as a genetic modifier for HD.

4.3 S18Y polymorphism in the UCHL1 gene

Former studies suggested that the UCHL1 gene influences the age at onset in HD patients. We studied in 946 HD patients the correlation between the CAG repeat expansion and the S18Y polymorphism. We analysed the effect of the S18Y polymorphism together with the expanded CAG repeat (HD CAG) on the HD age at onset. Though this analysis

resulted in a significant p value for S18Y alone (p=0.008) and for its interaction with the CAG repeat (p=0.007). By defining different age classes, we identified a threshold in the number of CAG repeats. The S18Y showed a significant effect on the age at onset of HD patients (p=0.001) when the HD CAG repeats were divided in two subgroups with short alleles <50 repeats and long alleles >50 repeats. The SS and SY genotype act protectively in the presence of longer CAG repeats with >50 units. (**Table 2)**

Table 2

Mean age-at-onset (AAO) in different subsamples of HD patients

	Number of patients	Mean AAO (SD)
<50 CAG repeats		
Genotype SS	524	47,6 ±12,0
Genotype SY	241	48,6 ± 12,2
Genotype YY	45	48,0 ± 11,1
>50 CAG repeats		
Genotype SS	91	28,8 ± 8,2
Genotype SY	39	29,3 ± 9,9
Genotype YY	6	20,3 ± 11,5

SD Standard deviation, S wild type allele (serine), Y mutant allele (tyrosine).

4.4 International Cooperative Ataxia Rating Scale ICARS

Among the 156 patients there were 72 females and 84 males. The average age at onset was 49.2 ± 14.1 years (range: 13-80 years). The duration of the disease was 10.6 ± 6.9 years (range:0-37). The ICARS score was correlated with the Barthel Index and the ataxia disease stages as external criteria. From the 156 patients 94 were in the first stage, 36 in the second stage and 26 in the 3rd stage. The ataxia stage of the controls was 0.

The time needed for the ICARS scale was 21.3 ± 7.1 minutes. The ICARS score was correlated higher with disease stages (p=0.001). The ICARS scores were in the stages 1, 2 and 3 28.8 ± 11.7 (range:7-57), 45.8 ± 15.6 (range:17-81) and 66.0 ± 9.9 (range: 49-87). For the controls it was 2.1 ± 1.4.

4.5 Scale for the Assessment and Rating of Ataxia (SARA)

Analysis showed high interrater reliability of SARA total score and the items related to gait, stance, fast alternating hand movements and heel shin slide, in contrast the other items were less successful (<0.8). The second trial showed, that the mean SARA score was 15.9 ± 8.5 (range: 1.5 to 40). The mean SARA score of the control group was 0.4 ± 1.1 (range: 0-

7.5). 79% of the controls had a score of 0.

Linearity of SARA was tested by a regression analysis of video ratings of 11 patients with global assessments as a reference. The SARA ratings and the differences between measures fitted a linear model (SARA: $p<0.0001$; $r2 = 0.98$; differences: $p<0.0001$; $r2= 0.72$).

The SARA score increased with the disease stage ($p<0.001$). The SARA score was closely correlated with the Barthel Index ($r=-0.80$; $p<0.0001$) and UHDRS-IV ($r=-0.89$; $p<0.0001$), while it had only a weak correlation with disease duration ($r=0.34$; $p<0.0002$). Further analysis showed that the correlation between SARA score and disease duration was stronger, when only SCA-1 ($r=0.74$; $p<0.003$) or SCA3 patients ($r=0.59$; $p<0.002$) were considered. This was not found for SCA2 ($r=0.08$; $p>0.68$) and SCA6 ($r=0.26$; $p>0.28$).

4.6 Non-ataxia symptoms

Demographical data, SARA and INAS scores are shown in **Table 3**. The age at onset was similar by SCA1, SCA2 and SCA3 but it was considerably higher in SCA6.

Table 3

Demographic, genetic, and clinical characteristics of the study population

	SCA1	SCA2	SCA3	SCA6
No.	117	163	139	107
M/F	71/46	75/88	73/66	58/49
Age, y	46.3 ± 12.2	46.3 ± 13.3	48.8 ± 11.8	64.9 ± 11.0
Age at onset, y	37.0 ± 10.6	34.9 ± 12.7	37.1 ±11.4	54.5 ± 10.2
Disease duration, y	9.5 ± 5.5	11.3 ± 6.5	11.6 ± 5.9	10.4 ± 6.4
SARA	15.6 ± 9.1	15.8 ± 8.0	15.1 ± 8.6	15.0 ± 6.7
INAS count	5.0 ± 2.1	4.6 ± 2.1	5.2 ± 2.5	2.0 ± 1.7

The disease severity was similar in all groups. Correlation between SARA score, repeat lengths, age, age at onset and duration of the disease are shown in **Table 4**.

Table 4

Correlation between SARA score, repeat number, age, age at onset, and duration of disease

	SCA1		SCA2		SCA3		SCA6	
	R	p	R	p	R	p	R	p
Repeat length expanded allele	0.02	0.815	0.38	<0.001	0.35	<0.001	-0.20	0.043
Repeat length normal allele	-0.15	0.117	-0.04	0.580	0.17	0.056	0.00	0.993
Age, y	0.28	0.002	-0.09	0.252	0.08	0.334	0.38	<0.001
Age at onset, y	-0.03	0.773	-0.28	<0.001	-0.16	0.054	0.08	0.400
Duration of disease, y	0.65	<0.001	0.39	<0.001	0.49	<0.001	0.53	<0.001

The distribution of non-ataxia symptoms are shown in **Table 5**. Pyramidal signs are most frequent in SCA1, whereas motor symptoms indicating peripheral nerve involvement were most frequent in SCA2. Myoclonus, rigidity, chorea/dyskinesia, dystonia and resting tremor were rare in all groups with the exception of dystonia which was found in 23.9% of all patients with SCA3.

Table 5

Frequency of nonataxia symptoms

	SCA1	SCA2	SCA3	SCA6
Hyperreflexia	67.5%	13.2%	40.1%	21.9%
Areflexia	17.9%	64.4%	57.8%	23.8%
Extensor plantar	50.5%	31.0%	41.9%	2.0%
Spasticity	59.3%	8.9%	44.4%	13.6%
Paresis	22.4%	14.4%	24.8%	5.7%
Muscle atrophy	29.1%	22.5%	39.0%	10.7%
Fasciculations	39.1%	38.3%	37.0%	2.8%
Myoclonus	4.3%	13.7%	4.4%	0.0%
Rigidity	1.7%	7.4%	10.3%	5.7%
Chorea/dyskinesia	6.8%	6.8%	10.1%	1.9%
Dystonia	12.8%	14.2%	23.9%	4.7%
Resting tremor	6.8%	14.9%	3.6%	1.9%
Sensory symptoms	62.4%	68.4%	65.6%	48.0%
Urinary dysfunction	35.0%	40.4%	45.6%	31.1%
Cognitive impairment	21.5%	25.9%	19.3%	10.5%
Brainstem oculomotor signs	75.9%	90.7%	80.6%	60.2%

To identify factors that determine the occurrence of specific non-ataxia symptoms we performed a logistic regression analysis for each symptom with repeat length of the expanded allele, age at onset, age, disease duration and SARA score as independent variables. For SCA1, this analysis showed that higher age was associated with urinary disfunction and cognitive impairment. In SCA2, longer repeats and earlier age at onset increased the likelihood of muscle atrophy and brainstem oculomotor signs. Longer repeats were further associated with chorea/dyskinesia and dystonia. In SCA3, larger repeats were associated with spasticity and hyperreflexia. In SCA6, the likelihood of a number of symptoms increased with age at onset, disease duration and age. The INAS count was similar in SCA1, SCA2, SCA3, but considerably lower in SCA6. The INAS count increased with disease duration in all groups.

4.7 SCA Composite Functional Index SCAFI

The only difference of clinical characteristics was older age in SCA6 patients (65.2 y vs. 47.0 y in SCA1-3). More than half (58%) of the patients performed the walking test unassisted. Performance in 9HPT was faster with the dominant than with the non-dominant hand. All subtests Z-scores as well as SCAFI showed a strong negative linear correlation with SARA that was highest for SCAFI and lowest for PATA. SCAFI could be calculated in 383 cases (93% of all participants). Effects of age, gender or cognitive impairment were analysed as possible factors that influence the test results. A moderate effect of age on 8MW and SCAFI was seen, but remained significant only for SCA1 and SCA6 (Pearson r=-0.206; p=0.042). Correlations with the interview-based assessment of functional independence (UHDRS-IV) were similarly high and positive, i.e., higher functional independence was associated with better performance for all three functional measures.

5. DISCUSSION

5.1 Suicide in Huntington's disease patients

Suicide among patients with HD is more frequent than in the general Hungarian population, the latter incidence being 45/100.000 inhabitants/year. Suicide accounted for 10% of all deaths in the 396 HD patients in this study. The families in this study are from the Western part of Hungary, while the greatest incidence of suicide has been reported for the eastern part of the country. The higher suicide rate among men may reflect the more effective methods utilized.

5.2 Genetic analysis of modifier genes

None of the studied gene polymorphism showed a significant correlation with the age at onset of the HD. Recent evidence from a transgenic mouse model supports the hypothesis that environmental factors contribute to the variation in the age at onset of the disease.

5.3 S18Y polymorphism in the UCHL1 gene

We found a significant effect of this polymorphism for the beginning of the disease in the HD patients. We found a protective effect of the SS and the SY genotypes in the presence of longer CAG repeats than 50 units. The age at onset was 28.8 years (SD8.2) and 29.3 years (SD9.9) for the SS and SY genotypes but only 20.3 years (SD11.5) for the YY genotype. The polymorphism shows a protective effect on the age at onset for repeats >50, patients with the genotypes YY have their first symptoms 8 years former than other genotypes.

5.4 International Cooperative Ataxia Rating Scale ICARS

A former study evaluated the properties of ICARS in a smaller patient group. During the analysis they used videotapes to test-retest reliability. Our analysis was more complicated, using Barthel Index, which measures activities of daily living and disease stages as external

criteria. Ideally, a rating scale is short, easy to apply and does not give rise to erroneous ratings. In the case of the ICARS scale, the redundant and overlapping nature of several items gave rise to a considerable number of contradictory ratings, but it could be reduced by practice like the time needed for performance.

Ideally, the rating results of ICARS should be determined by four factors that correspond to the four subscales. Our analysis showed, that the rating results were determined by four different factors which did not coincide with the ICARS subscales, therefore questioning the justification of ICARS subscore analysis in clinical trials.

5.5 Scale for the Assessment and Rating of Ataxia (SARA)

SARA was easy to administer and required less than 15 minutes per patients. The time is considerably shorter than the time required to complete ICARS. Most importantly, SARA results were determined by a single factor, which indicate that SARA measures cerebellar ataxia. Separate analysis of the four most common genotypes showed that the correlation between SARA score and disease duration was better when only patients with SCA1 or SCA3 were considered. That was not found for SCA2 and SCA6.

5.6 Non-ataxia symptoms

Onset of the disease was similar to other studies. The aim of our study was to identify factors that determine the severity in the most common SCA subtypes. To assess disease severity, we used the SARA scale, which was validated in large clinical trials. Disease duration was positively correlated with SARA score in all groups. A comparison of the regression curves revealed that progression was fastest in SCA1 and somewhat slower in SCA2, SCA3 and SCA6. For SCA6, age at onset was the only factor other than disease duration that contributed to the SARA score. For SCA1, SCA2 and SCA3 the most important contributory factor was the repeat length of the expanded allele.

Our data add further weight to the view that SCA1, SCA2, SCA3 share a number of biologic properties whereas SCA6 is not only distinct in that it starts later and has less non-ataxia symptoms, but also in that its phenotype is more determined by age than by disease-related factors.

5.7 SCA Composite Functional Index SCAFI

The correlations with disease progression for the Composite Functional Index were stronger than for the any subtest alone. SCAFI showed a linear decline over the whole range of disease severity. The proposed SCAFI has favourable properties to assess patients with spinocerebellar ataxia.

6. SUMMARY OF THE RESULTS AND CONCLUSIONS

1. Suicide accounted for 10% of all deaths in the HD patients, the rate was higher among men. The methods of suicide were different from those of the general population.

2. None of the genes GRIK2, TBP, BDNF and ZDHHC17 could be identified as a genetic modifier for HD.

However, we found a significant effect of the S18Y polymorphism in the UCHL1 gene on the age at onset when longer alleles of at least 50 CAG repeats are present.

3. ICARS has a number of intrinsic problems that clearly limits its usefulness.

4. The SARA is a reliable and valid measure of ataxia, making it an appropriate primary outcome measure for clinical studies.

5. Progression was fastest in SCA1, this is in line with clinical observations. In the SCA6 model, age at onset was the only factor that contributed to the SARA score. In SCA1, SCA2 and SCA3 SARA score and disease duration were the strongest predictors of the INAS count. In SCA6, only age at onset contributed to the INAS count. INAS captures other aspects than SARA, it might be useful as an additional outcome measure in clinical studies.

6. The correlation with disease progression was stronger for SCAFI than for the any subtest alone.

7. LIST OF PUBLICATIONS

The thesis is based on the following publications

1. ***Baliko L,*** Csala B, Czopf J. Suicide in Hungarian Huntington's disease patients. *Neuroepidemiology* 2004;23(5):258-260. IF: 1,758

2. Metzger S, Bauer P, Tomiuk J, Laccone F, Didonato S, Gellera C, Soliveri P, Lange HW, Weirich-Schwaiger H, Wenning GK, Melegh B, Havasi V, ***Baliko L,*** Wieczorek S, Arning L, Zaremba J, Sulek A, Hoffman-Zacharska D, Basak AN, Ersoy N, Zidovska J, Kebrdlova V, Pandolfo M, Ribai P, Kadasi L, Kvasnicova M, Weber BH, Kreuz F, Dose M, Stuhrmann M, Riess O. The S18Y polymorphism in the UCHL1 gene is a genetic modifier in Huntington's disease. *Neurogenetics* 2006;7(1):27-30. IF: 4,250

3. Schmitz-Hubsch T, Tezenas du Montcel S, ***Baliko L***, Boesch S, Bonato S, Fancellu R, Giunti P, Globas C, Kang JS, Kremer B, Mariotti C, Melegh B, Rakowicz M, Rola R, Romano S, Schols L, Szymanski S, van de Warrenburg BP, Zdzienicka E, Durr A, Klockgether T. Reliability and validity of the International Cooperative Ataxia Rating Scale: a study in 156 spinocerebellar ataxia patients. *Mov Disord* 2006;21(5):699-704. IF: 3,323

4. Schmitz-Hubsch T, du Montcel ST, ***Baliko L,*** Berciano J, Boesch S, Depondt C, Giunti P, Globas C, Infante J, Kang JS, Kremer B, Mariotti C, Melegh B, Pandolfo M, Rakowicz M, Ribai P, Rola R, Schols L, Szymanski S, van de Warrenburg BP, Durr A, Klockgether T,

Fancellu R. Scale for the assessment and rating of ataxia: development of a new clinical scale. *Neurology* 2006;66(11):1717-20. Erratum in: Neurology. 2006;67(2):299. IF: 5.690

5. Metzger S, Bauer P, Tomiuk J, Laccone F, Didonato S, Gellera C, Mariotti C, Lange HW, Weirich-Schwaiger H, Wenning GK, Seppi K, Melegh B, Havasi V, ***Baliko L,*** Wieczorek S, Zaremba J, Hoffman-Zacharska D, Sulek A, Basak AN, Soydan E, Zidovska J, Kebrdlova V, Pandolfo M, Ribai P, Kadasi L, Kvasnicova M, Weber BH, Kreuz F, Dose M, Stuhrmann M, Riess O. Genetic analysis of candidate genes modifying the age-at-onset in Huntington's disease. *Hum Genet* 2006;120(2):285-292. IF: 3,662

6. Schmitz-Hübsch T, Giunti P, Stephenson DA, Globas C, ***Baliko L,*** Saccá F, Mariotti C, Rakowicz M, Szymanski S, Infante J, van de Warrenburg BP, Timmann D, Fancellu R, Rola R, Depondt C, Schöls L, Zdzienicka E, Kang JS, Döhlinger S, Kremer B, Melegh B, Filla A, Klockgether T. SCA Functional Index – a useful compound performance measure for spinocerebellar ataxia. *Neurology* 2008;71(7):486-492. IF: 6,014

7. Schmitz-Hübsch T, Coudert M, Bauer P, Giunti P, Globas C, ***Baliko L***, Filla A, Mariotti C, Rakowicz M, Charles P, Ribai P, Szymanski S, Infante J, van de Warrenburg BP, Dürr A, Timmann D, Boesch S, Fancellu R, Rola R, Depondt C, Schöls L, Zdienicka E, Kang JS, Döhlinger S, Kremer B, Stephenson DA, Melegh B, Pandolfo M, di Donato S, du Montcel ST, Klockgether T. Spinocerebellar ataxia type 1, 2, 3 and 6: Disease severity and nonataxia symptoms. *Neurology* 2008;71(13):982-989. IF: 6,014

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