Table 1. Demographic and clinical features of 7 SCA patients with full *C9orf72* repeat expansions

Patient Number	SCA Type	CAG Repeats Number (Small/Large)	*C9orf72* Repeats	Gender	Age of Onset	SARA	Mental Status	Motor neuron		
								Fasciculation	Weakness	Reflexes of Extrer
1	1	30/44	5/>30	Woman	38	10.5	MoCA 25/30	Present	None	Hyperreflexia in bic patellar and Achille
2	1	30/42	10/>30	Woman	45	0.5	MoCA 30/30	Present	None	Hyperreflexia in bic patellar and Achille
3	1	30/42	10/>30	Woman	52	8.5	MoCA 25/30	Present	None	Hyperreflexia in bic patellar and Achille
4	2	22/39	6/>30	Man	35	24	Not evaluated	None	None	Areflexia in biceps, patellar and Achille
5	2	22/39	2/>30	Woman	40	24.5	Poor in serial 7s	Mild in tongue/ face and four limbs	Mild in four limbs	Areflexia in biceps, patellar and Achille
6	3	28/70	2/>30	Woman	48	30.5	Not evaluated	Moderate in tongue and face	Mild in four limbs	Areflexia in biceps, hyperreflexia in pat and Achilles
7	6	11/23	8/>30	Man	59	12.5	Not evaluated	None	None	None

Abbreviations: SCA: spinocerebellar ataxias, SARA: scale for ataxia rating and assessment, MoCA: Montreal Cognitive Assessment, Serial 7s: seria

Supplemental Table 1. Frequency of full repeat *C9orf72* expansions in SCA patients and normal American population.

	Normal American population	SCA1	SCA2	SCA3	SCA6
***C9orf72* expansions ≥ 31, n**	1659	48	56	108	58
***C9orf72* expansions ≤ 30, n**	0	3	2	1	1
Total, n	1659	51	58	109	59
***p* value [a]**	-	0.000	0.001[a]	0.062[a]	0.034[a]

American normal controls are from the reference 5.

[a]Fisher exact test

Supplemental Table 2. Baseline features of SCA participants with normal (≤ 7) and intermediate (8-30) *C9orf72* repeat expansions

	SCA 1 n = 48		p-value[d]	SCA 2 n = 56		p-value[d]	SCA 3 n = 108		p-value[d]	SCA 6 n = 58		p-value[d]
	Normal	Intermediate		Normal	Intermediate		Normal	Intermediate		Normal	Intermediate	
Sample size, n (%)	28 (58.3)	20(41.7)		35 (62.5)	21 (37.5)		68 (63.0)	40 (37.0)		31 (53.4)	27 (46.6)	
Age at first visit, y	49.8 ± 14.4	50.3 ± 11.5	0.888[b]	51.0 ± 14.0	47.3 ± 12.5	0.329[b]	53.0 ± 12.1	48.6 ± 12.3	0.075[b]	68.2 ± 10.0	62.1 ± 12.2	0.038[b]
Gender, M : W	17 : 11	10 : 10	0.461[a]	17:18	13:8	0.333[a]	30 : 38	21 : 19	0.399[a]	16:15	14:13	0.986[a]
Age of onset, y	39.9 ± 12.9	39.7 ± 9.5	0.943[b]	36.6 ± 13.5	35.0 ± 10.9	0.646[b]	39.9 ± 12.5 Median = 40.0	37.2 ± 11.6 Median = 38.5	0.339[c]	52.7 ± 10.3	49.7 ± 11.0	0.290[b]
Disease duration, y	9.9 ± 8.3 Median = 8.0	10.6 ± 6.3 Median =7.5	0.508[c]	15.0±9.1	12.3 ± 6.0	0.245[b]	13.4 ± 8.0 Median = 12.0	11.6 ± 7.9 Median = 11.0	0.204[c]	15.5 ± 11.8 Median = 12.0	12.3± 9.7 Median = 9.0	0.285[c]
Expanded CAG repeats	46.4 ± 4.1 Median = 47.0	46.6 ± 5.0 Median = 46.5	0.966[c]	40.1 ± 3.1 Median = 39.0	40.9 ± 3.7 Median = 40.0	0.298[c]	70.4 ± 3.9 Median = 71.0	72.0 ± 4.3 Median = 72.0	0.111[c]	22.4 ± 1.1 Median = 22.0	22.3 ± 0.7 Median = 22.0	0.656[c]
SARA scores	12.6 ± 8.4 Median = 12.25	15.9 ± 7.7 Median = 14.25	0.177[c]	17.5 ±6.9	17.7 ±7.3	0.939[b]	15.3 ± 8.2 Median = 14.5	15.4± 10.1 Median = 13.25	0.929[c]	16.1 ± 7.1	14.3 ± 7.8	0.343[b]
PHQ-9 scores	5.5 ± 6.3 Median = 3.0	8.0 ± 7.1 Median = 7.5	0.178[c]	5.2 ± 5.0 Median = 4.0	5.4 ± 5.7 Median = 3.0	0.931[c]	8.0± 6.1 Median = 6.0	5.7 ± 4.1 Median = 5.5	0.101[c]	6.7± 6.0 Median = 6.0	7.7± 6.4 Median = 6.0	0.594[c]

Abbreviations: PHQ-9= The 9-item Patient Health Questionnaire, SARA = Scale for Assessment and Rating of Ataxia, SCA = Spinocerebellar Ataxia.

Values represent mean ± standard deviation or number, and for variables with non-normal distribution, the median is reported as well.

[a] Chi-square test

[b] 2 independent samples t-test

[c] 2 independent samples Mann-Whitney U test

[d] A Bonferroni correction was made to adjust for multiple comparisons, p< 0.007 were considered significant (7 tests in each subtypes)

Supplemental Table 3. Longitudinal SARA and PHQ-9 scores of normal and intermediate *C9orf72* repeat expansion in GEE models

	Regression coefficients of SARA score					
	Age of first visit (yrs)	**Sex**	**Expanded CAG repeats**	**Allele type of *C9orf72***	**Visit time**	**Allele type of *C9orf72* × Visit time**
SCA1	0.62****	4.71**	1.42****	2.66	0.95*	-0.04
SCA2	0.42****	-1.40	1.87****	0.64	0.40	-0.84
SCA3	0.67****	-0.82	1.88****	0.14	0.37*	-0.02
SCA6	0.11	-1.68	0.71	2.12	0.23	0.96

	Regression coefficients of PHQ-9 score					
	Age of first visit (yrs)	**Sex**	**Expanded CAG repeats**	**Allele type of *C9orf72***	**Visit time**	**Allele type of *C9orf72* × Visit time**
SCA1	0.08	-0.37	0.25	2.16	0.32	-1.90***
SCA2	0.01	-1.35	-0.12	1.54	1.00***	-0.63
SCA3	0.26***	2.41	0.66*	-5.49****	-0.38	3.48****
SCA6	-0.12*	-2.65	-1.23*	-1.01	0.30	-1.72*

Abbreviations: GEE = generalized estimating equation, PHQ-9 = The 9-item Patient Health Questionnaire, SARA = Scale for Assessment and Rating of Ataxia, SCA = Spinocerebellar Ataxia.

*p <0.05, **p < 0.01, ***p < 0.005, ****p <0.001

Sex: women = 0, men = 1

Allele type of *C9orf72*: normal allele = 0, intermediate allele = 1

Visit time: time order every 6 month during the two-year follow up

C9orf72 Repeat Expansions as Genetic Modifiers for Depression in Spinocerebellar Ataxias

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The genetic interactions between pathological repeat expansions have been of major interests in neurodegenerative disorders. Recently, pathogenic *C9orf72* repeat expansions, a main genetic cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia and pathogenic *ATXN2* repeat expansions, the causative gene for spinocerebellar ataxia (SCA) type 2, are reported to coexist in a single family with ataxia.[1] Therefore, this observation raises an interesting possibility that *C9orf72* repeat expansions could be genetic modifiers in CAG-repeat SCAs and might influence the disease progression.

Therefore, we studied 277 patients with SCA1, 2, 3 and 6 from the Clinical Research Consortium for Spinocerebellar Ataxias (CRC-SCA) cohort,[2] and we determined the *C9orf72* repeat length as previously described.[3] The Scale for Assessment and Rating of Ataxia (SARA) and the 9-item Patient Health Questionnaire (PHQ-9) were used to measure the severity of ataxia and depression, respectively. We studied the rate of ataxia and depression progression using generalized estimating equation to test whether the intermediate repeats of *C9orf72* were associated with ataxia or depression progression in SCAs. As described previously, full repeat expansions of *C9orf72* were defined as ≥ 31 hexanucleotide repeats whereas intermediate repeat expansions were 8-30.[3-5]

We identified seven patients (3 out of 51 SCA1; 2 out of 58 SCA2; 1 out 109 SCA3; 1 out of 59 SCA6) with pathogenic *C9orf72* repeat expansions. None of the 7

cases had motor neuron disease, but they had various degrees of motor neuron signs (**Table 1**). Compared to cognitively normal control (The original paper cites 1039 Europeans and 620 African American) population,[5] the frequencies of expanded *C9orf72* repeats in our cohort were significantly higher in SCA1, 2, and 6 but not in SCA3 (**Supplemental table 1**). Forty percent of SCA patients carry intermediate *C9orf72* repeat expansions, and the demographic and clinical features of SCA subjects with normal and intermediate alleles of *C9orf72* are shown in **Supplemental table 2**. Intermediate *C9orf72* repeat expansions did not influence the rate of ataxia progression but were associated with different rates of depressive symptom progression in SCA1, 3, and 6 (SCA1: $\beta = -1.90$, $p < 0.005$; SCA3: $\beta = 3.48$, $p < 0.001$; SCA6: $\beta = -1.72$, $p < 0.05$; **Supplemental table 3**).

In the present study, we identified patients of SCA1, 2, 3, and 6 who also carry pathogenic *C9orf72* repeat expansions. Intermediate *C9orf72* repeat expansions might influence the non-motor symptom (i.e. depression) progression in SCAs. Our study highlights the genetic interactions between repeat expansions.

The presence of CAG repeat expansions could interfere with the DNA repair process,[6] which may destabilize *C9orf72* repeat expansions and explain the co-existence of *C9orf72* repeat expansions and expanded CAG repeats. Since cerebellar pathology could be found in *C9orf72*-linked ALS, the presence of *C9orf72* repeat expansions might affect polyglutamine aggregates preferentially in the cerebellum or brainstem structures implicated in depression.

In conclusion, our study provides supporting evidence that repeat expansions of

C9orf72 may be genetic modifiers in SCAs, and perhaps ataxia patients in general.[7] Therefore, the interplay of repeat expansions in two different loci may lead to diverse clinical phenotypes in degenerative cerebellar ataxia.

Author contributions

Ms. Figueroa: study concept and design, statistical analysis and interpretation, writing the manuscript, critical revision of the manuscript for important intellectual content.

Dr. Gan: study concept and design, statistical analysis and interpretation, critical revision of the manuscript for important intellectual content.

Dr. Perlman: acquisition of data.

Dr. Wilmot: acquisition of data.

Dr. Gomez: acquisition of data.

Dr. Schmahmann: acquisition of data.

Dr. Paulson: acquisition of data.

Dr. Shakkottai: acquisition of data.

Dr. Ying: acquisition of data.

Dr. Zesiewicz: acquisition of data.

Dr. Bushara: acquisition of data.

Dr. Geschwind: acquisition of data.

Dr. Xia: acquisition of data.

Dr. Subramony: study concept and design, acquisition of data, study supervision.

Dr. Ashizawa: study concept and design, acquisition of data, critical revision of the

manuscript for important intellectual content, study supervision.

Dr. Pulst: study concept and design, acquisition of data, critical revision of the manuscript for important intellectual content, study supervision.

Dr. Kuo: study concept and design, acquisition of data, analysis and interpretation, critical revision of the manuscript for important intellectual content.

Disclosure: Dr. Zesiewicz has served as a clinical advisor for Steminent Biotherapeutics, and she has received travel reimbursement from the department of neurology at University of Southern Florida; has received travel reimbursement for a Biohaven Pharmaceuticals meeting. Dr. Zesiewicz has served on the editorial board for Neurodegenerative Disease Management and Tremor and other Hyperkinetic Movements, and has received research support for her division for approximately 20 clinical trials for Parkinson's disease, Friedreich's ataxia, and spinocerebellar ataxias. Dr. Zesiewicz's division is a site in a multi-site trial of Parkinson's disease patients with the LRRK2 mutation and is sponsored by the National Institutes of Health but funded by Emory University. The rest of authors report no conflicts of interest.

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Table 1. Demographic and clinical features of 7 SCA patients with full *C9orf72* repeat expansions

Patient Number	SCA Type	CAG Repeats Number (Small/Large)	*C9orf72* Repeats	Gender	Age of Onset	SARA	Mental Status	Motor neuron deficits				
								Fasciculation	Weakness	Reflexes of Extremities	Plantar Reflex	Spasticity
1	1	30/44	5/>30	Woman	38	10.5	MoCA 25/30	Present	None	Hyperreflexia in biceps, patellar and Achilles	Extensor	Mild in lower and upper limbs
2	1	30/42	10/>30	Woman	45	0.5	MoCA 30/30	Present	None	Hyperreflexia in biceps, patellar and Achilles	Flexor	Not evaluated
3	1	30/42	10/>30	Woman	52	8.5	MoCA 25/30	Present	None	Hyperreflexia in biceps, patellar and Achilles	Flexor	Mild in lower limbs
4	2	22/39	6/>30	Man	35	24	Not evaluated	None	None	Areflexia in biceps, patellar and Achilles	Extensor	Moderate in lower limbs, mild in upper limbs
5	2	22/39	2/>30	Woman	40	24.5	Poor in serial 7s	Mild in tongue/ face and four limbs	Mild in four limbs	Areflexia in biceps, patellar and Achilles	Flexor	None
6	3	28/70	2/>30	Woman	48	30.5	Not evaluated	Moderate in tongue and face	Mild in four limbs	Areflexia in biceps, hyperreflexia in patellar and Achilles	Extensor	Moderate in four limbs
7	6	11/23	8/>30	Man	59	12.5	Not evaluated	None	None	None	None	None

Abbreviations: SCA: spinocerebellar ataxias, SARA: scale for ataxia rating and assessment, MoCA: Montreal Cognitive Assessment, Serial 7s: serial sevens subtraction test,

Supplemental Table 1. Frequency of full repeat *C9orf72* expansions in SCA patients and normal American population.

	Normal American population	SCA1	SCA2	SCA3	SCA6
***C9orf72* expansions ≥ 31, n**	1659	48	56	108	58
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Total, n	1659	51	58	109	59
***p* value [a]**	-	0.000	0.001[a]	0.062[a]	0.034[a]

American normal controls are from the reference 5.

[a]Fisher exact test

Supplemental Table 2. Baseline features of SCA participants with normal (≤ 7) and intermediate (8-30) *C9orf72* repeat expansions

	SCA 1 n = 48		p-value[d]	SCA 2 n = 56		p-value[d]	SCA 3 n = 108		p-value[d]	SCA 6 n = 58		p-value[d]
	Normal	Intermediate		Normal	Intermediate		Normal	Intermediate		Normal	Intermediate	
Sample size, n (%)	28 (58.3)	20(41.7)		35 (62.5)	21 (37.5)		68 (63.0)	40 (37.0)		31 (53.4)	27 (46.6)	
Age at first visit, y	49.8 ± 14.4	50.3 ± 11.5	0.888[b]	51.0 ± 14.0	47.3 ± 12.5	0.329[b]	53.0 ± 12.1	48.6 ± 12.3	0.075[b]	68.2 ± 10.0	62.1 ± 12.2	0.038[b]
Gender, M : W	17 : 11	10 : 10	0.461[a]	17:18	13:8	0.333[a]	30 : 38	21 : 19	0.399[a]	16:15	14:13	0.986[a]
Age of onset, y	39.9 ± 12.9	39.7 ± 9.5	0.943[b]	36.6 ± 13.5	35.0 ± 10.9	0.646[b]	39.9 ± 12.5 Median = 40.0	37.2 ± 11.6 Median = 38.5	0.339[c]	52.7 ± 10.3	49.7 ± 11.0	0.290[b]
Disease duration, y	9.9 ± 8.3 Median = 8.0	10.6 ± 6.3 Median =7.5	0.508[c]	15.0±9.1	12.3 ± 6.0	0.245[b]	13.4 ± 8.0 Median = 12.0	11.6 ± 7.9 Median = 11.0	0.204[c]	15.5 ± 11.8 Median = 12.0	12.3± 9.7 Median = 9.0	0.285[c]
Expanded CAG repeats	46.4 ± 4.1 Median = 47.0	46.6 ± 5.0 Median = 46.5	0.966[c]	40.1 ± 3.1 Median = 39.0	40.9 ± 3.7 Median = 40.0	0.298[c]	70.4 ± 3.9 Median = 71.0	72.0 ± 4.3 Median = 72.0	0.111[c]	22.4 ± 1.1 Median = 22.0	22.3 ± 0.7 Median = 22.0	0.656[c]
SARA scores	12.6 ± 8.4 Median = 12.25	15.9 ± 7.7 Median = 14.25	0.177[c]	17.5 ±6.9	17.7 ±7.3	0.939[b]	15.3 ± 8.2 Median = 14.5	15.4± 10.1 Median = 13.25	0.929[c]	16.1 ± 7.1	14.3 ± 7.8	0.343[b]
PHQ-9 scores	5.5 ± 6.3 Median = 3.0	8.0 ± 7.1 Median = 7.5	0.178[c]	5.2 ± 5.0 Median = 4.0	5.4 ± 5.7 Median = 3.0	0.931[c]	8.0± 6.1 Median = 6.0	5.7 ± 4.1 Median = 5.5	0.101[c]	6.7± 6.0 Median = 6.0	7.7± 6.4 Median = 6.0	0.594[c]

Abbreviations: PHQ-9= The 9-item Patient Health Questionnaire, SARA = Scale for Assessment and Rating of Ataxia, SCA = Spinocerebellar Ataxia.

Values represent mean ± standard deviation or number, and for variables with non-normal distribution, the median is reported as well.

[a] Chi-square test

[b] 2 independent samples t-test

[c] 2 independent samples Mann-Whitney U test

[d] A Bonferroni correction was made to adjust for multiple comparisons, p< 0.007 were considered significant (7 tests in each subtypes)

Supplemental Table 3. Longitudinal SARA and PHQ-9 scores of normal and intermediate *C9orf72* repeat expansion in GEE models

Regression coefficients of SARA score

	Age of first visit (yrs)	Sex	Expanded CAG repeats	Allele type of *C9orf72*	Visit time	Allele type of *C9orf72* × Visit time
SCA1	0.62****	4.71**	1.42****	2.66	0.95*	-0.04
SCA2	0.42****	-1.40	1.87****	0.64	0.40	-0.84
SCA3	0.67****	-0.82	1.88****	0.14	0.37*	-0.02
SCA6	0.11	-1.68	0.71	2.12	0.23	0.96

Regression coefficients of PHQ-9 score

	Age of first visit (yrs)	Sex	Expanded CAG repeats	Allele type of *C9orf72*	Visit time	Allele type of *C9orf72* × Visit time
SCA1	0.08	-0.37	0.25	2.16	0.32	-1.90***
SCA2	0.01	-1.35	-0.12	1.54	1.00***	-0.63
SCA3	0.26***	2.41	0.66*	-5.49****	-0.38	3.48****
SCA6	-0.12*	-2.65	-1.23*	-1.01	0.30	-1.72*

Abbreviations: GEE = generalized estimating equation, PHQ-9 = The 9-item Patient Health Questionnaire, SARA = Scale for Assessment and Rating of Ataxia, SCA = Spinocerebellar Ataxia.

*p <0.05, **p < 0.01, ***p < 0.005, ****p <0.001

Sex: women = 0, men = 1

Allele type of *C9orf72*: normal allele = 0, intermediate allele = 1

Visit time: time order every 6 month during the two-year follow up