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ORIGINAL PAPER

Spinocerebellar Ataxias in Brazil—Frequencies and Modulating Effects of Related Genes

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Abstract This study describes the frequency of spinocerebellar ataxias and of CAG repeats range in different geographical regions of Brazil, and explores the hypothetical role of normal CAG repeats at *ATXN1*, *ATXN2*, *ATXN3*, *CACNA1A*, and *ATXN7* genes on age at onset and on neurological findings. Patients with symptoms and family history compatible with a SCA were recruited in 11 cities of the country; clinical data and DNA samples were collected. Capillary electrophoresis was performed to detect CAG lengths at SCA1, SCA2, SCA3/MJD, SCA6, SCA7, SCA12, SCA17, and DRPLA associated genes, and a repeat primed PCR was used to detect ATTCT expansions at SCA10 gene. Five hundred forty-four patients (359 families) were included. There were 214 SCA3/MJD families (59.6 %), 28 SCA2 (7.8 %), 20 SCA7 (5.6 %), 15 SCA1 (4.2 %), 12 SCA10 (3.3 %), 5 SCA6 (1.4 %), and 65 families without a molecular diagnosis (18.1 %). Divergent rates of SCA3/MJD, SCA2, and SCA7 were seen in regions with different ethnic backgrounds. 64.7 % of our SCA10 patients presented seizures. Among SCA2 patients, longer *ATXN3* CAG alleles were associated with earlier ages at onset ($p<0.036$, linear regression). A portrait of SCAs in Brazil was obtained, where variation in frequencies seemed to parallel ethnic differences. New potential interactions between some SCA-related genes were presented.

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Introduction

The spinocerebellar ataxias (SCAs) are neurodegenerative disorders that share a complex neurological presentation of an ataxia of adult onset and an autosomal dominant inheritance. To date, at least 41 genetic loci have been related to a given SCA [1, 2]. Many of them result from nucleotide repeat expansions, and seem to be the most prevalent ones. Seven SCAs (SCA1, SCA2, SCA3/MJD, SCA6, SCA7, SCA17, and dentatorubro-pallidoluysian atrophy, DRPLA) are caused by a CAG repeat expansion within the coding region, producing an extended polyglutamine tract in the mutant protein. In SCA12, the disease is also the result of an expanded (CAG)n, but in the 5′ of the *PPP2R2B* gene. In SCA8, there is an expanded CTG-CAG repeat, located in both an untranslated region of the *ATXN8OS* gene and a short ORF in the overlapping *ATXN8* gene. A pentanucleotide expansion is implicated in SCA10, caused by a tract of hundreds of ATTCT repeats in an intron of the *ATXN10* gene [1, 2]. Other intronic expansions have been reported in the *BEAN* gene in SCA31 and in the *NOP56* gene in SCA36 [3, 4].

Each type of SCA is individually rare worldwide, with largely variable frequencies among populations (for a review, see [5]). There are few data on the prevalence of individual types of SCAs. In contrast, relative frequencies of each form among large diagnostic series of patients ascertained through diagnostic centers are available to be compared. The most frequent SCA worldwide is Machado–Joseph disease (SCA3/MJD), followed by SCA2, SCA1, and SCA6. SCA10 has been diagnosed only in North and South America so far—namely, in Mexico [6], Brazil [7–9], Argentina [10], and Venezuela [11]. SCA36 corresponds to 6 % of SCAs in Galicia [12], whereas SCA31 could be the fourth most common in some regions of Japan [13]. Other SCAs may be rare everywhere.

The search for relative frequencies of SCAs in Brazilian series of cases has been limited to Southern and Southeast regions. The largest series published to date included 114 and 104 families from Rio Grande do Sul [9] and Parana [14], where 84 and 48 % of them carried MJD. However, Brazil is a huge country, and these figures probably do not portrait correctly the epidemiology of SCAs in Brazil.

Each individual SCA presents phenotypic variations in age at onset (AO), neurological manifestations, and progression rate. In the case of SCAs due to expanded (CAG)n, the (CAG)n repeat length is responsible for 45–80 % of AO variation ([1, 15–21] among others). Other explanations for the clinical heterogeneity of SCAs have been sought for instance in intrafamilial factors [15, 22], candidate genes [18, 23, 24], and methylation patterns [25], with interesting but partial success.

The aims of this study were to describe the relative frequencies of SCA1, SCA2, SCA3/MD, SCA6, SCA7, SCA10, SCA12, SCA17, and DRPLA on different regions of Brazil, to

test the possible relationship of these diagnoses with normal CAG repeats range found in each geographical region, and to explore the hypothetical role of *ATXN1*, *ATXN2*, *ATXN3*, *CACNA1A*, and *ATXN7* normal alleles on AO and neurological findings of SCA2, SCA3/MJD, and SCA7 patients.

Methods

Ethics Statement

The present work has been approved by the Ethics Committee from the institution at which the work was performed—Comissão de Ética em Pesquisa do Hospital de Clínicas de Porto Alegre, which follows the Code of Ethics of the World Medical Association (Declaration of Helsinki) and the standards established by the author's Institutional Review Board and granting agency. We have obtained written informed consent from all participants involved in the study.

Patients

Patients' recruitment was based on local referrals to centers specialized in Neurogenetics (mainly Universitary) for wide catchment in 11 urban centers of Brazil (Fig. 1). In each center, a neurologist (OB, JLP, DZS, CG, HLJ, RMC, and LBJ) or clinical geneticist with experience in neurogenetics (FRV, MAFDL, MBPT, HYW, PFVM, ETP, and ER) evaluated cases and their families, and invited them to participate in this study. The inclusion criteria were the presence of ataxia of adult onset, with or without other neurological signs and symptoms, and of an autosomal dominant pattern of inheritance. Since both inclusion criteria should be met, no exclusion criteria were used. All data were collected prospectively. Age at onset (AO) was defined as that at which patient or a close relative noticed the beginning of first symptom (usually gait unbalance). Data such as age, gender, AO, disease duration, presence or absence of several neurological findings obtained from a conventional neurological examination (ataxia, nystagmus, ophthalmoparesis in general, dysarthria, dysphagia, fasciculations, pyramidal findings, absent muscular reflexes, sensory losses, visual loss, rigidity, dystonic

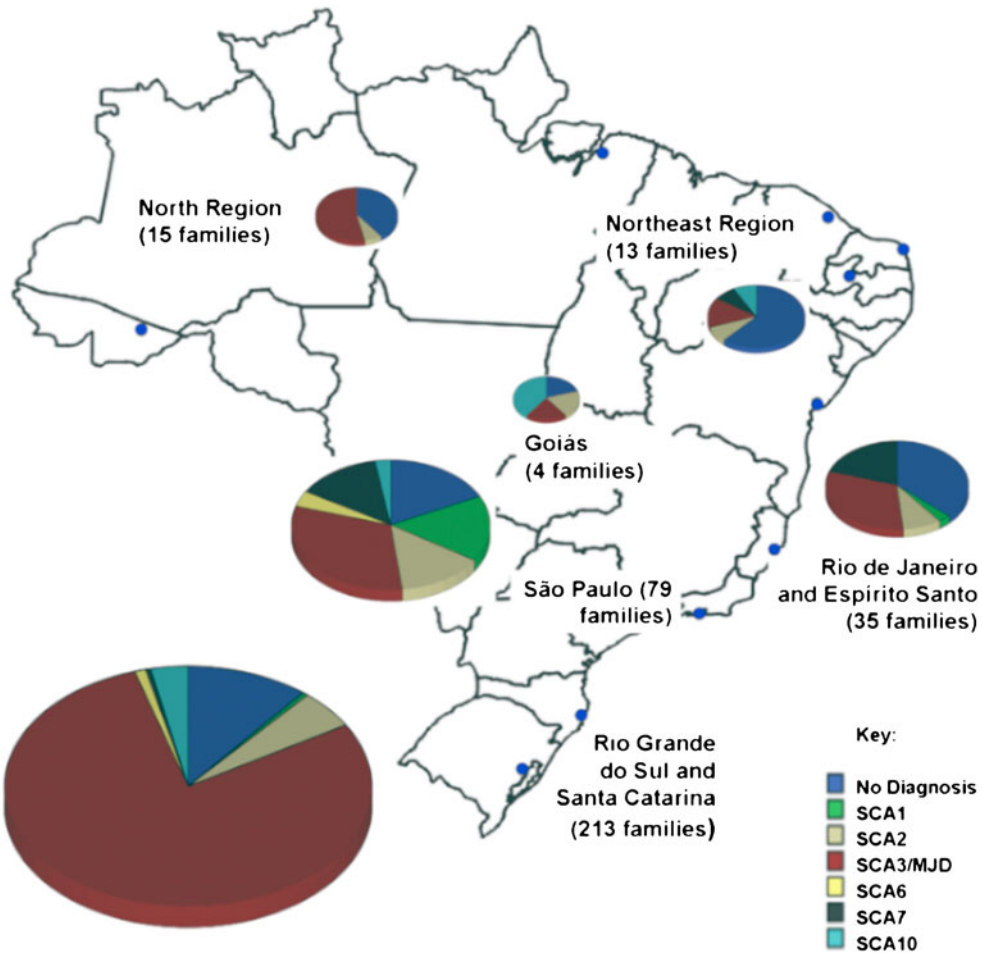

Fig. 1 Brazil: urban centers and regions where the present families were originated, and the relative frequencies of different diagnoses

movements, bradykinesia, tremor, seizures, and cognitive losses), as well as geographical origin, and family history, were collected through an online digital form. In each 1 of the 11 hospitals, the assistant physician collected demographic and neurological information and entered them into the electronic database, in our website. After consent, a blood sample was collected and then sent from the site of origin to the central laboratory, where the molecular analyses were performed.

Methods

Genomic DNA was obtained from peripheral blood by the salting out method [26]. Two different multiplex PCR were performed using fluorescence-labeled primers flanking the respective repeats. Following amplification, PCR products were separated on an ABI3130xl Genetic Analyzer. Expanded allele in the ATXN10 gene was detected by the repeat primed-PCR (TP-PCR) methodology as previously described [27]. Length of the expanded repeats at ATXN10 was not determined.

All patients diagnosed up to 2009 were studied by a gene-by-gene (step-by-step) approach—at first the *ATXN3* gene was studied, then, if no expansion was observed, *ATXN2* gene were tested, and so on. Since 2010, all DNA samples were tested by a three-panel approach based on fluorescent multiplex-PCR. The first panel included the simultaneous study of regions of interest at *ATXN1*, *ATXN2*, *ATXN3*, *CACNA1A*, and *ATXN7* genes. If no expansion was detected, samples were tested by a second panel, that included the regions of interest at *PPP2R2B*, *TBP*, and *ATN1* genes. Finally, if no expansion was detected by both panels, the *ATXN10* gene was then tested.

Patient characteristics are given as mean ± SD, range, and 95 % CI, when applicable. Categorical variables such as diagnosis, region of origin and presence or absence of neurological findings, were compared through Fisher exact test and logistic regression. CAG repeats in most loci, and disease duration in several diagnostic categories did not show a normal distribution on Shapiro-Wilk test. The remaining continuous variables were normal. Comparisons of ages at onset between diagnostic categories were performed through ANOVA test with contrasts. Normal CAG repeats distribution from different geographical origins of subjects were compared by Kruskal–Wallis test. Correlations between age at onset and the CAG repeats of the expanded and the normal alleles were tested with Spearman correlation test followed by linear regression model.

In those SCA2, SCA3/MJD, and SCA7 patients diagnosed from 2010 onwards, the presence or absence of a given neurological finding was tested according to the sets of the normal, CAG repeat alleles at *ATXN1*, *ATXN2*, *ATXN3*, *CACNA1A*, and *ATXN7* genes. Variables such as age, disease duration, and the causal expanded CAG repeat at each disease were also included in the model because all of them could influence the present phenotype. A logistic regression (binary logistic) was utilized to control for all these potential confounding factors, potentially related to the outcomes under study. CAG repeats at each given locus was subdivided into larger and shorter alleles. Given that shorter alleles did not show any association with dependent variables, they were excluded from the model. Ages at onset were also not included in this set, given its covariance with each of the expanded CAG repeats under study. Statistical significance was defined as $p<0.05$. All statistical tests were performed in PASW 18.

Results

Proportion of Diagnoses and Range of Normal Repeats Per Subject's Geographical Origin

As of March 2012, 544 patients from 359 SCA families were investigated: 354 (213 families) from Rio Grande do Sul and Santa Catarina States (in the South Region), 94 (79 families) from São Paulo State, 38 (35 families) from Rio de Janeiro and Espírito Santo States, 23 (15 families) from North Region, 30 (13 families) from Northeast Region, and 5 (4 families) from Central-Western Region. The first 270 earlier patients were tested in a gene-by-gene approach; the former 274 were studied by the three-panels approach, described in the "Methods" section.

SCA3/MJD was the most common SCA, accounting for 214 families (59.6 %) and for 337 patients (62.5 %) recruited in the present case series. The obtained diagnoses are described in Fig. 1 and in Table 1. Diagnoses described here include former families reported by our group (114 families in [9] and three additional families in [28]) [9, 28].

Sixty-five Brazilian families with SCA (18.2 %) remained without diagnosis. In these families, SCA1, SCA2, SCA3/MJD, SCA6, SCA7, SCA10, SCA12, SCA17, and DRPLA have been excluded ("no diagnosis", or ND families). ND families were even more common in Northeast (61.5 %, Table 1) and North (40 %) regions, and in Rio de Janeiro state (37.1 %; $p<0.05$, exact Fisher test).

Normal CAG and ATTCT repeat lengths at these loci were obtained from each region of Brazil; the majority of these data were obtained by the three-panels approach, described in the "Methods" section. The repeat ranges in each Brazilian region did not show any peculiar pattern, nor were related to specific proportions of SCA diagnoses (Table 2).

Associations Between Clinical Characteristics and Molecular Results

General clinical data such as age at onset and disease duration were obtained in 518 out of the 544 patients of the present series, and are presented in Table 3. A detailed neurological description was obtained in only 389 out of the original 544 patients and is presented in Table 4. It is worth to remind that

Table 1 Total number and percent of SCA families found, according to diagnoses and to geographical origin in Brazil

	Region of Origin						Total
	South Region (Rio Grande do Sul and Santa Catarina States)	São Paulo	Rio de Janeiro and Espírito Santo	North Region (Pará and Acre States)	Northeast Region (Rio Grande do Norte, Paraíba, Bahia and Ceará States)	Central-Western Region (Goiás State)	
No diagnosis (ND)	23	14	13	6	8	1	65
	10.8 %	17.7 %	37.1% *	40% *	61.5% *	25 %	18.1%
SCA1	1	13	1	0	0	0	15
	0.5 %	16.5%*	2.9 %				4.2%
SCA2	11	11 *	3	1	1	1	28
	5.2 %	13.9%	8.6 %	6.7 %	7.7 %	25 %	7.8%
SCA3/MJD	167	25	11	8	2	1	214
	78.4% *	31.6 %	31.4 %	53.3 %	15.4 %	25 %	59.6%
SCA6	2	**3**	0	0	0	0	5
	0.9 %	3.8% *					1.4%
SCA7	1	11	**7**	0	1	0	20
	0.5 %	13.9% *	20 %*		7.7 %		5.6
SCA10	8	2	0	0	1	1	12
	3.7 %	2.5 %			7.7 %	25% *	3.3%
Total	213	79	35	15	13	4	359
	100 %	100 %	100 %	100 %	100 %	100 %	100 %

*$p<0.05$, Fisher exact test, adjusted standardized residuals

this analysis included only a subset of patients, and that we cannot rule out an ascertainment bias.

Ages at Onset

Ages at onset, disease duration and number of expanded repeats for each condition are shown in Table 3. SCA6 patients showed an older age at onset, whereas SCA2 and SCA7 patients showed an earlier age at onset than the overall group; and ND patients showed longer disease duration than the other diagnostic categories ($p<0.05$, ANOVA with contrasts).

Ages at onset were inversely related to length of the expanded repeats in those SCAs where this comparison was feasible—SCA1, SCA2, SCA3/MJD, SCA6, and SCA7 (Electronic Supplementary Material (ESM) Fig. 1). In SCA10, the effect of the expanded ATTCT repeat on the AO cannot be analyzed, since length of these repeats was not determined.

The hypothetical influence of CAG tracts in the normal range found at *ATXN1*, *ATXN2*, *ATXN3*, *CACNA1A*, and *ATXN7* loci on AO in 49 patients with SCA2, 102 patients with SCA3/MJD, and 32 patients with SCA7 were tested by linear regression test. Normal CAG tract found at the larger *ATXN3* allele was associated with AO in SCA2 patients ($r=-0.31$, $p<0.036$, linear regression, Fig. 2). In SCA3/MJD and SCA7, only the expanded repeat has influenced AO of the specific diagnostic category (as shown in Table 3 and ESM Fig. 1).

Neurological Findings

The proportions of several neurological findings were compared between the different categories: SCA1, SCA2, SCA3/MJD, SCA6, SCA7, SCA10, and ND patients. This analysis was done in the subset of 389 patients where the data were available.

Binary logistic regression was performed, with age and disease duration included in the model. Cox and Snell *R* square was presented when a trend ($p<0.1$) or an association ($p<0.05$) was found. Table 4 shows that dysarthria–dysphagia and amyotrophies were significantly more frequent in SCA3/MJD than in other SCAs ($p<0.05$).

Normal CAGn Repeats As Possible Modifiers of the SCA Phenotypes

A preliminary search for a possible modifier role of the normal CAG repeats at diverse loci on the neurological findings was performed in a subgroup of 183 patients. Diseases under study were SCA3/MJD (including 102 patients), SCA2 (49 patients), and SCA7 patients (32 patients), in the present cohort.

Table 2 Number of CAG repeats found in each locus, and classified as normal, expanded and uncertain alleles. CAG repeats in normal alleles were also shown in specific regions, where the related SCA was more common than in other geographical areas

	ATXN1 (SCA1)	*ATXN2* (SCA2)	*ATXN3* (SCA3/ MJD)	*CACNA1A* (SCA6)	*ATXN7* (SCA7)	*PPP2R2B* (SCA12)	*TBP* (SCA17)	*ATN1* (DRPLA)	*ATXN10* (*SCA10*)
	CAG repeats								ATTCT repeats
Number of individuals (alleles) studied	273 (546)	285 (570)	481 (962)	275 (550)	280 (560)	88 (176)	88 (176)	88 (176)	88 (176)
Expanded repeats, *m* (range)	46.7 (39–60)	42.1 (34–67)	75.45 (65–89)	24.3 (22–26)	50.4 (37–73)	–	–	–	NM
n	20	51	344	11					23
CAG repeats of uncertain interpretation or reduced penetrance	One allele with 36 repeats [a]	Four alleles with 33 repeats[b]	None	One allele with 19 repeats[c]	None.	None.	None.	NA	NA
Normal repeats, *m* (range)	30 (19–35)	22.08 (11–30)	22.27 (7–38)	11 (4–17)	10 (7–14)	11.64 (9–19)	34.6 (27–38)	13 (2–19)	14 (7–20)
n	526	516	618	539	528	176	176	176	153
Normal CAG repeats on the subpopulation with the highest number of affected families, *m* (range)	29.6 (20–35)	22.19 (17–30)	21.85 (7–38)	11.41 (6–17)	10 (7–14)	–	–	–	–
n	170	176	362	182	66				
	São Paulo	São Paulo	Rio Grande do Sul	São Paulo	Rio de Janeiro				

NA not applicable, *NM* not measured

[a] One *ATXN1* allele with 36 CAG repeats was found in a SCA3/MJD patient (with 30/36 repeats at *ATXN1* and 27/79 repeats at *ATXN3*)

[b] Four siblings from Rio Grande do Sul State with SCA2 and parkinsonian phenotype had their short *ATXN2* alleles with 33 repeats (their *ATXN2* genotypes were 33/34, 33/34, 33/34, and 33/43). They have been previously reported [62]

[c] One *CACNA1A* allele with 19 CAG repeats was found in a SCA6 patient of Japanese ancestry (with 19/23 repeats) who inherited the allele with 23 repeats from her father (13/23 repeats).` His mother was already deceased and his maternal family has no history of ataxic symptoms

These patients were included in the three-panels approach, described in the "Methods" section, and therefore were tested for the CAG repeats of interest in this section. In other words, this subset included only those individuals investigated after 2010. For this reason, we cannot rule out an ascertainment bias related to recent years.

Disease duration, chronological age, the large normal CAG allele at *ATXN1*, *ATXN2*, *ATXN3*, *CACNA1A*, and *ATXN7*

Table 3 Age at onset, disease duration and number of expanded repeats for each diagnostic category

	N	Disease duration	Age at onset	Expanded CAG repeat	Correlation between age at onset and expanded CAG repeat
		Mean±SD (95 % CI)		Mean (range)	R^2 **
No diagnosis (ND)	58	10.8±7.3 (8.9–12.8)*	36.6±15.4 (32.5–40.7)	–	–
SCA 1	19	6.1±4.7 (3.8–8.4)	37.1±11.2 (31.7–42.5)	47 (41–60)	0.70
SCA 2	49	10.1±7.7 (7.9–12.3)	29.7 ±10.6 (26.7–32.8)*	41 (34–67)	0.65
SCA 3/ MJD	328	8.21±6 (7.5–8.8)*	34±11.2 (32.8–35.2)	75 (67–89)	0.55
SCA 6	11	10.5±6.5 (6.2–14.9)	45.4±12.6 (36.9–53.8)*	24 (22–26)	0.66
SCA 7	30	7.7±5.2 (5.7–9.6)	25.5±11.4 (21.2–29.7)*	50 (38–73)	0.80
SCA 10	23	12.9±7.8 (9.4–16.4)	33.8±9.8 (29.5–38)	–	–
Total	518	8.8±6.4 (8.2–9.3)	33.7±12 (32.7–34.8)	–	–

*$p<0.05$, ANOVA with Tukey;
**$p<0.05$, Spearman correlation test

Table 4 Associations found between neurological signs and specific SCAs, controlled for disease duration and age at examination

	Was that related to DD, in the overall sample?	Was that related to age, in the overall sample?	Was that related to diagnosis?							
			Diagnosis (number of cases)							
			ND	SCA1	SCA2	SCA3/ DMJ	SCA6	SCA7	SCA10	Total
			46	9	37	247	9	21	20	389
			In percent							
Ataxia	ns	ns	100 %	100 %	100 %	100 %	100 %	100 %	100 %	99.5 %
			46	9	37	247	9	21	20	387
Alterations in ocular movement	p<0.007[a]	p<0.048 [a]	35 %	44 %	43 %	65 %	33 %	71 %	70 %	59 %
			16	4	16	161	3	15	14	(229)
Nystagmus	ns	ns	50 %	33 %	22 %	81 %	67 %	48 %	85 %	69 %
			23	3	8	201	6	10	17	(268)
Palpebral retraction	ns	ns	11 %	22 %	3 %	30%*	0 %	14 %	0 %	22 %
			5	2	1	73		3		84
Dysarthria and/or dysphagia	ns	ns	85 %	89 %	76 %	91%**	67 %	90 %	95 %	89 %
			39	8	28	226	6	19	19	345
Pyramidal findings	ns	p<0.001*	54 %	89 %	32 %	75 %	33 %	81 %	80 %	69 %
			25	8	12	186	3	17	16	267
Absent reflexes	p<0.001*	p<0.001*	26 %	0 %	43 %	26 %	0 %	0 %	10 %	24 %
			12		16	64			2	94
Amyotrophy	p<0.012*	ns	9 %	0 %	3 %	11% **	0 %	0 %	0 %	8 %
			4		1	28				33
Sensory losses	ns	ns	26 %	11 %	43 %	60 %	33 %	28.5 %	25 %	49 %
			12	1	16	149	3	6	5	192
Visual loss	ns	p<0.02*	11 %	0 %	8 %	20 %	11 %	90 %	31.3 %	21 %
			5		3	49	1	19	6	83
Dystonic movements	ns	p<0.001*	19.5 %	11 %	3 %	27 %	0 %	9.5 %	5 %	21 %
			9	1	1	67		2	1	81
Bradykinesia	p<0.006*	ns	22 %	0 %	30 %	36 %	0 %	0 %	45 %	30 %
			10		11	88			9	118
Rigidity	ns	ns	17 %	33 %	14 %	17 %	0 %	0 %	5 %	15 %
			8	3	5	41			2	58
Tremor	ns	p<0.032*	26 %	0 %	32 %	6 %	55.5 %	0 %	31.3 %	13 %
			12		12	14	5		6	49
Cognitive losses	p<0.005*	ns	19.5%*	0 %	22%*	3 %	0 %	5 %	10 %	7 %
			9		8	8		1	2	28
Seizures	ns	ns	6.5 %	11 %	0 %	0.8 %	0 %	5 %	65 %	5 %
			3	1		2		1	13	20

[a] *t* test

*$p<0.1$, binary logistic, Cox and Snell *R* square; **$p<0.05$, binary logistic, Cox and Snell *R* square (diagnosis, age of onset and disease duration as determinants of the neurological finding)

loci, as well as the expanded CAG allele, were included in the model.

At first sight, in SCA3/MJD patients, larger *ATXN2* CAG alleles seemed to be associated with abnormalities of ocular movement, nystagmus, and dystonic movements (ESM Fig. 2); and larger *CACNA1A* alleles seemed to be associated with palpebral retraction and absent reflexes (ESM Fig. 2). However, these trends have lost significance on binary logistic (Cox and Snell *R* square, not significance (ns)). In a larger sample of SCA3/MJD (the formers plus other 128 patients), as

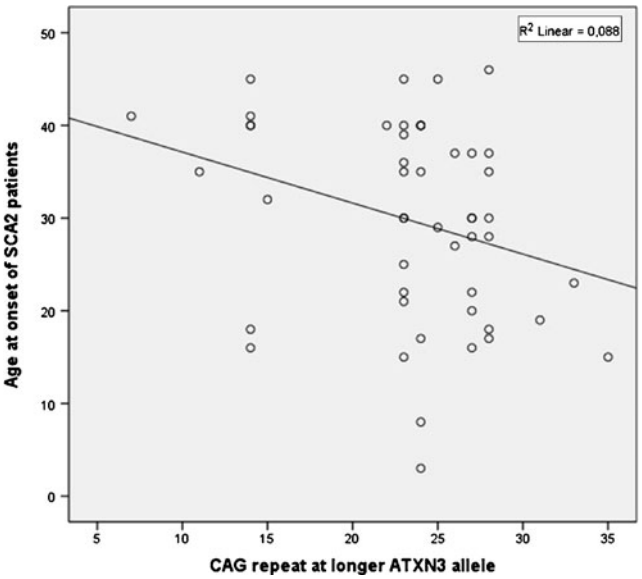

Fig. 2 *ATXN3* gene might modulate SCA2 phenotype. Correlations between the normal CAG repeats at the large *ATXN3* allele and age at onset in 49 SCA2 patients of the present series

expected, larger expanded CAG repeats at the *ATXN3* allele were associated with dystonic movements, whereas shorter expanded CAG repeats were associated with absent reflexes.

Similarly, an apparent relation between longer CAG alleles at *ATXN1* and abnormalities of ocular movements in SCA2 did not achieve significance (Cox and Snell *R* square, ns; binary logistic, ESM Fig. 3).

Discussion

We were able to expand the portrait of Brazilian SCAs by covering geographic regions that were not formerly included in frequency studies. As worldwide, SCAs are rare in Brazil and SCA3/MJD continued to be the most frequent SCA reported in our country. Variation in the frequency of the other SCAs might be related to differences in populations of origin. Moreover, we were able to extend the clinical information on some diseases such as SCA10, and to address new potential interactions between some SCA-related genes, which were picked up by associations with some neurological findings.

Rates

Several factors are involved in the variation of the SCA frequencies per population, such as the frequency of large normal (unstable) alleles and of haplotypes more prone to expansion. Probably the main reasons are founder effects or genetic drift. Table 5 lists some papers on this field and exemplifies the large variation in observed frequencies of SCAs according to countries, regions or ethnic groups. For instance, in South Africa, SCA7 is the most common form among Africans, while SCA1 surpasses it in mixed and non-African individuals (Table 5). Globally, SCA3/MJD and SCA2 predominate. Exceptions to this generalization do exist, such as SCA8 in Finland, SCA6 in Japan, SCA1 in Serbia, SCA7 in Africans from South Africa, and, perhaps, SCA10 among Mexicans (see references in Table 5).

Similarly, in a mixed population such as in Brazil, large variations in frequencies of different SCAs would be expected. Brazil is the fifth largest country in the world. His 190 million inhabitants occupy different regions with quite diverse population ancestries. Although all regions have Amerindian ancestry, the North region shows the largest influence of the Amerindian root. In contrast, the South was mostly settled by western European immigrants—at least six million Europeans came to this part of Brazil since the nineteenth century. Slavery lasted until the same century and explains the strong African presence. This mixed population is reflected in the studies of mtDNA haplotypes of White Brazilians, among others [29, 30]. In this scenario, the dynamics of an increasing population as well as several founder effects could be playing a role in changing the frequencies of certain diseases. For instance, a rapid increasing population could be theoretically related to variations in the range of the CAG repeats and to the appearance of de novo expansions [31]. However, we did not find any population trend towards an increase in range of normal CAG repeats that could be related to any specific SCA with an increased frequency (Table 2). It rests to test the role of founders in a relatively recent population like ours, descending from European and African founders in the last 500, and from Amerindians in the last 10–15,000 years.

This report confirms previous results that SCA3/MJD is by far the most common SCA found in Brazilian families, with some minor differences in the observed proportions of the next most common SCAs—SCA2, SCA7, SCA1, and SCA10 [9, 14, 32]. South Brazil was first settled by Azoreans in the eighteenth century, and we suggested that SCA3/MJD patients from South Brazil descend from these migrants [32]. However, SCA3/MJD was also the most frequent SCA found in other Brazilian states, for where there was no major Azorean but mainland Portuguese migration, and further studies are required to clarify their ancestry.

SCA2 was the second most common SCA in our country, at least according to the present series. It was mostly found in São Paulo State, the most populated urban region of the country and with the most heterogeneous ancestry. We speculate whether this finding could be related to the ancestral composition of that region. São Paulo received around one million migrants from Italy [33], where SCA2 is the most frequent SCA [34, 35]. However, haplotype studies so far have shown that SCA2 has arisen from several independent-

Table 5 Relative frequencies of diverse SCAs according to country of origin, in some representative case series. Stratifications according to regions, in Brazil, or ethnic groups, in South Africa, were also presented

Country strata	Families	Proportions of diagnoses											References
	n	SCA1	SCA2	SCA3/MJD	SCA6	SCA7	SCA8	SCA10	SCA12	SCA17	DRPLA	Other	
Australia	88	16	6	12	17	2	–	–	–	–	0	47	[49]
China	120	6	7	49	3	1	–	–	–	–	0	34	[50]
Finland	49	4	2	0	2	12	18	–	–	–	0	62	[51]
Germany	77	9	10	42	22	–	–	–	–	–	–	17	[17]
India	77	16	25	3	0	3	–	–	6	–	0	47	[52]
Italy	183	21	24	1	1	1	1	0	0	1	1	50	[35]
Japan	330	6	2	28	26	1	–	–	–	–	7	30	[42]
Korea	87	0	13	5	7	0	–	–	–	–	3	72	[53]
Mexico	108	0	45	12	0	7	0	14	0	3	0	18	[54]
Netherlands	145	6	7	28	15	8	–	–	–	–	–	36	[55]
Portugal	45	2	2	58	0	4	–	0	0	0	11	22	[56]
Serbia	38	34	13	0	0	0	0	–	0	0	0	53	[57]
South Africa													[58]
Black	83	4	36	1	0	59	–	–	–	–	–	–	
Non-Black	82	70	17	7	5	1							
Spain	72	6	15	15	1	3	–	–	–	–	1	59	[59]
Taiwan	81	1	11	32	0	1	–	–	–	–	–	55	[60]
USA	178	6	15	21	15	4	5	–	–	–	–	34	[61]
Brazil													Present series
Total	359	4	8	60	1	6	–	3	0	0	0	18	
Regions													
South	213	0.5	5	78	1	0.5	–	4	0	0	0	11	
Southeast	114	12	12	32	3	16	–	0	0	0	0	24	
Northeast	13	0	8	15	0	8	–	8	0	0	0	61	
North and Central	19	0	11	47	0	0	–	5	0	0	0	37	

For a comprehensive review on the epidemiology of SCAs, we suggest the paper by [8], among others

origin mutations [5], and the origin of Brazilian cases waits for a more precise elucidation.

Although the numbers are still not large, differences in the rate of SCA7 in the diverse populations of Brazil are impressive. SCA7 families were mainly from Sao Paulo and Rio de Janeiro. Rio de Janeiro was the capital of the country during most of the Portuguese domination period. During this colonial period, slavery was quite prominent in this region. It is relevant to mention that although SCA7 may be one of the less frequent forms of dominant ataxia worldwide [5], a large family from Northeast Brazil contributed to the SCA7 gene discovery [36]. In South Africa, SCA7 occurred exclusively in indigenous Black African patients and seems to have a higher incidence than the rest of the world [37, 38]. We speculate here if some Brazilian SCA7 families would share an *ATXN7* allele of African ancestry.

Results presented here also expand our knowledge about SCA10 among Brazilians [9–11, 28, 39, 40]. SCA10 was the fifth most common SCA in the present sample, being detected in 3.3 % or 12 of the present families (three of them have already been described in [8, 9, 28]). Originally described in an American family of Mexican origin [41], SCA10 accounts for almost 15 % of all dominant ataxias in Mexicans. Further reports of SCA10 patients from Argentinian and Venezuelan kindreds [10] and Venezuela [11], supported the hypothesis of an Amerindian origin.

The present study was able to screen for nine genes related to SCAs, but we are aware that at least other 32 genetic loci have been identified as causally related to this phenotype. We did not find any case of SCA12, SCA17, or DRPLA. In contrast, 62 SCA families (18.2 %) remained with unknown diagnosis (ND). The majority of ND families came from Northeast (64 %, Table 1) and North (42.8 %) regions, and from Rio de Janeiro (35.5 %). Although different clinical suspicion criteria could be operating according to the region of origin, we rather think that these results point to an important amount of some SCAs other than those we have studied in this report.

Clinical Findings and Expanded Repeats

Several findings confirmed many previous observations on SCAs—namely, age at onset of SCA1, SCA2, SCA3/MJD, and SCA6. Correlations between ages at onset and CAG repeats also confirmed early, classical findings, the exception being probably SCA7, where the obtained R^2 of 0.77 was higher than in other populations [20, 21].

Among our SCA10 patients, epilepsy was far more common than in former Brazilian cases [39]; actually, our rates resembled those found in Mexican patients [6]. The present series included 23 individuals with a DD of 12.9 years and living up to 3,500 km away from each other. Data on neurological examination were obtained in 20 of them, and in 13 (65 %) individuals, generalized tonic–clonic seizures or combinations of myoclonic, complex partial, and generalized tonic–clonic seizures occurred. In contrast, in a large series of 80 SCA10 patients living near Curitiba (in Santa Catarina State), epilepsy was found only in 3.75 % of the cases with a mean DD of 15 years [39]. Our series included only one case from Santa Catarina State: unfortunately, the patient was lost of follow-up and a detailed neurological information was not obtained. Seizures were present in six and absent in three of our families; in the other three families, this information was lacking. Our positive families included epileptic and non-epileptic individuals (there was intrafamilial phenotypic heterogeneity). The present six families with epilepsy were found in all Brazilian regions under study. The three "epilepsy-free" families belonged to Rio Grande do Sul and São Paulo States, where the majority of families under study were living. No relation between disease duration and seizures was found.

Former case series have presented the phenotypic contrasts between different SCAs [17, 42, 43]. An attempt to improve our results was made by controlling confounding differences such as chronological ages and disease duration—both independent variables that could partially determine the neurological manifestations. By doing so, some differences in the proportions of neurological findings were obtained (Table 4). However, we are aware that prospective studies on natural history are the best design to test these differences [44–46].

Normal CAG Repeats As Candidates to Modify SCA Phenotypes

In the present study, logistic regression has detected some relationships between CAG repeats at some loci and clinical manifestations in some SCAs.

In SCA3/MJD patients, a direct relationship between the expanded CAG repeat at *ATXN3* and dystonic movements was shown while an inverse correlation was seen with absent reflexes. These results are in agreement with classical findings of SCA3/MJD, and might confirm the quality of our data and the rationale of the present approach. The novelty was the suggested relationships of the normal CAG repeat length at *ATXN3* gene with the age at onset of SCA2.

Potential relationship between *ATXN2* and *ATXN3* genes (or their proteins ataxin-2 and ataxin-3) has long been studied. For instance, normal ataxin-2 can be detected in the pathogenic inclusions of SCA3/MJD patients, and, likewise, normal ataxin-3 localizes to the inclusions formed in SCA2 patients [47]. In a Drosophila model, toxicity and neurodegeneration induced by pathogenic forms of *ATXN3* depend on the normal activity of the wild ataxin-2 of the fly [48]. Curiously, we were unable to reproduce here our previous finding that fasciculations in SCA3/MJD correlate with the CAG repeat in *ATXN2* normal alleles [23]. Differences between our previous and the present observations might be due to differences between clinical protocols, to sample size, or by chance.

In summary, SCA3/MJD was confirmed to be the most frequent SCA in Brazilians, followed by SCA2, SCA7, and SCA10. Variations of the CAG repeats, either in the normal as well as in the expanded range, were similar to those found elsewhere. Ages at onset and neurological manifestations were all expected, with the possible exception of the presence of seizures among Brazilian patients with SCA10. And although the CAG expansion in a single allele of the responsible gene is sufficient for the determination of each SCA, our observations suggest that some interaction of the *ATXN3* gene on AO in SCA2 might occur. These results deserve confirmation in a second population, and deeper studies in cellular and animal models.

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Conflict of Interest The authors state that there is no potential conflict of interest

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