The Cerebellum (2019) 18:188–194
https://doi.org/10.1007/s12311-018-0977-7

ORIGINAL PAPER

Selective Forces Related to Spinocerebellar Ataxia Type 2

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Published online: 15 September 2018

Abstract

Spinocerebellar ataxia type 2 (SCA2) is caused by an unstable expanded CAG repeat tract (CAGexp) at *ATXN2*. Although prone to selective forces such as anticipation, SCA2 frequency seems to be stable in populations. Our aim was to estimate reproductive success, segregation patterns, and role of anticipation in SCA2. Adult subjects from families with molecular diagnosis provided data about all his/her relatives. Affected and unaffected sibs older than 65.7 years of age were used to estimate reproductive success and segregation patterns. Twenty-one SCA2 families were studied, including 1017 individuals (164 affected) who were born from 1840 to 2012. The median number of children of the non-carriers and carriers, among 99 subjects included in the reproductive success analysis, were 2 and 3 ($p < 0.025$), respectively. Therefore, the reproductive success of carriers was 1.5. There were 137 non-carriers (59.6%) and 93 carriers (40.4%) ($p = 0.04$), among subjects included in the segregation analysis. Age at onset across generations pointed to anticipation as a frequent phenomenon. We raised evidence in favor of increased reproductive success related to the carrier state at *ATXN2*, and segregation distortion favoring normal alleles. Since majority of normal alleles analyzed carried 22 repeats, we propose that this distortion segregation can be related to the high frequency of this allele in human chromosomes.

Keywords Anticipation · CAG repeats · Reproductive success · Segregation distortion · SCA2 · Spinocerebellar ataxia type 2

Introduction

Spinocerebellar ataxia type 2 (SCA2) is an autosomal dominant cerebellar ataxia caused by an unstable expansion of a CAG repeat tract (CAGexp) at the ataxin-2 gene, *ATXN2* [1]. Normal *ATXN2* CAG tracts range from 12 to 32–3 repeats, the most frequent of them being the allele carrying 22 CAG repeats, representing 75–90% of alleles in several populations [2–4]. The CAG expansion introduces an abnormally large polyglutamine (polyQ) tract in the protein, giving rise to toxic effects that lead to neuronal function and survival [5].

Very disabling, SCA2 is associated to a progressive cerebellar syndrome. SCA2 is a late-onset disease with initial symptoms usually appearing when affected subjects are in

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s12311-018-0977-7) contains supplementary material, which is available to authorized users.

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their 30s [6]. As observed in other polyQ diseases, a strong negative correlation was observed between age at onset (AO) and length of CAGexp [7]. The expansion is unstable upon cellular divisions, and offsprings might carry shorter or longer expansions than CAGexp of the transmitting parent. Therefore, the disease might start at a later or earlier age than in the transmitting parent. Although rare, very late onset [1] and even lack of family history [8] have been reported in subjects carrying short expanded alleles: contractions can be behind those cases. Anticipation has been much more frequently observed, with several reports of carriers starting the disease during childhood [9–11]. Although previous analyses stated that male and female transmissions were related to increases of the CAGexp in the offspring [12, 13], sibships were not completely genotyped in the same report, and lack of information from asymptomatic children might have biased these data.

The overall impact of intergenerational instability of CAGexp transmission remains unknown. In case that further CAGexp expansions upon transmissions are really so frequent, a reduction in SCA2 frequency in populations would be expected, since severe anticipations would be associated to increased neurological deficits as well as to reduced fertility. However, there is no evidence pointing to a decline in SCA2 frequency. In contrast to this negative selection force, positive selective forces might be operating in SCA2 transmission simultaneously, as reported previously in other polyQ disorders, such as Huntington disease, spinocerebellar ataxia type 1, and spinocerebellar ataxia type 3/Machado-Joseph disease [14–17]. Positive selective forces would counterbalance negative forces, maybe keeping expanded alleles in the genetic pool. In order to contribute to the understanding of these forces, our aim was to evaluate genetic fitness—or the reproductive success—, segregation patterns, and anticipation phenomena in SCA2.

Methods

Adult subjects belonging to Brazilian families from Rio Grande do Sul, São Paulo, Acre, and Rio Grande do Norte states, with molecular diagnosis of SCA2 performed in our Institution, were invited to participate in the present survey, which was carried out from July 2016 to July 2017. Informants provided data about all his/her relatives in order to build their family trees. Variables under study were investigated from all relatives belonging to the included families: date of birth, date of death, gender, kinship relationships, order of birth, symptomatic status, and number of children. Age at onset (AO) of first symptom was obtained from the symptomatic subjects.

The genetic fitness (or the reproductive success) of SCA2 carriers was considered to be

$w = \frac{W\ \text{affected}}{W\ \text{unaffected}}$, where W was the median number of children of affected and unaffected subjects.

Since the genotype was not available for most family members under study, phenotype was used to help classify relatives of patients as affected or unaffected subjects. Individuals with ataxic manifestations and/or with a molecular diagnosis comprised the affected subjects. Asymptomatic subjects older than two standard deviations up from the mean age at onset of the entire cohort were considered the unaffected individuals. In accordance, the affected subjects under the present analysis were those older than the same age cutoff (see "Results" section). In order to reduce the impact of potential ascertainment and memory bias for analysis of reproductive success, the reproductive history of the individual included and of his/her affected parent should be entirely known.

Segregation analysis compared number of affected with that of unaffected sibs, children of one affected parent. In this analysis, again, all sibs (affected and unaffected) should be older than at least two standard deviation from the mean AO of the entire cohort.

Anticipation was considered the difference between AO of the transmitting parent and AO of the child. This delta-AO was zero when both the affected parent and child had the same AO. Cases of anticipation were defined when delta-AO was equal or greater than 1. Similarly, cases of later onset of the disease in the offspring were defined when delta-AO was equal or less than − 1.

Most group characteristics showed non-parametric distributions and were compared using Mann-Whitney U, Spearman's rank-order correlation, or chi-square test. Since AO showed normal distribution, ANOVA was used to estimate AO distributions among CAGexp groups. A $p < 0.05$ was chosen to support assumptions made. For statistical analysis, we used PASW Statistics 17 software.

The study protocol was approved by the ethics committee of our institution (registered as CAAE: 56652416.2.0000.5327 at the Brazilian National platform, Plataforma Brasil). All patients gave informed consent to participate in the study.

Results

Thirty-one Brazilian families received a molecular diagnosis of SCA2 from our Institution since 2001, and 21 agreed to participate in this study. One family declined to participate, and 9 were lost. Data from 1017 relatives were retrieved: 164 were symptomatic subjects and 84 of them were still alive. AO showed a normal distribution (one-sample Kolmogorov-Smirnov test 0.153), with a mean (SD) of 36.5 (14.6) years of age. Molecular analyses were available

from 50 individuals, reported elsewhere [18]. Normal and expanded alleles showed an average ± SD (range) of 23 ± 3 (22–33) and 40 ± 4 (34–53) CAG repeats. Three symptomatic subjects inherited an *ATXN2* allele with 33 repeat from the normal parent: their *ATXN2* genotypes were 33/34, 33/34, and 33/43 CAG repeats; this family was already described [19]. Since alleles with 33 repeats were seen more than once in the Brazilian population, they were considered normal [20]. The $(CAG)_{22}$ allele corresponded to 78% of normal alleles. Reproductive success, segregation, and anticipation analyses included only subjects older than 65.7 years (2 SD from the mean AO)—i.e., born before 1951. Symptomatic subjects were considered affected, while the remaining ones were considered unaffected.

Thirty-seven affected and 62 unaffected subjects were included in the analysis of reproductive success. Their median number of children were 3 and 2, respectively ($p < 0.021$,). SCA2 reproductive success was then estimated as $w = \frac{3}{2} = 1.50$ (Fig. 1a). Number of children of affected individuals showed a direct correlation to AO (rho = 0.49, $p < 0.001$) (Fig. 1b). The reproductive success changed according to AO of the affected subject, becoming lower than that of healthy sibs in subjects with AO earlier than 20 years.

Mean (SD) age of the affected parent at birth of their children was 31.45 (8.39) years for men and 28.24 (8.01) years for women ($p = 0.66$, Mann-Whitney *U* test), while AO was 37.07(15.73) and 35.93 (13.55) for affected fathers and mothers ($p = 0.79$, Mann-Whitney *U* test), respectively. Great majority of affected children (92%) were born before the parent's onset of symptoms (Supplemental Fig. 1).

One hundred thirty-nine sibs, children of symptomatic subjects, were included in the segregation analysis: 56 (40.4%) were affected while the remaining 83 (59.6%) were unaffected subjects (chi-square = 5.245, $p = 0.022$). Gender was explored and significant distortion was achieved among daughters (female offspring) of affected parents (Table 1).

AO was inversely related to CAGexp obtained from 49 subjects (rho = − 0.708, $p < 0.0001$, Spearman); each additional CAG repeat was associated to an average reduction of 1.57 years in the mean AO ($p < 0.003$, ANOVA). AO was also inversely related to date of birth obtained from symptomatic subjects, when all symptomatic subjects (rho = − 0.771, $p < 0.0001$) and when only those older than 2SD (rho = − 0.556, $p < 0.001$, Spearman) were analyzed (Fig. 2).

Finally, in relation to anticipation analysis, the average delta-AO between AO of affected transmitting parent and child was 10.70 ± 7.79 years. Delta-AO were 12 (55 to − 11) and 10 (35 to − 5) years, when the transmitting parents were fathers and mothers, respectively ($p = 0.052$, Mann-Whitney) (Supplemental Fig. 2).

Discussion

Three selective forces that potentially affect SCA2 recurrence on next generations were studied through phenotype analysis. SCA2 was associated to an increase in reproductive success (fitness) for those carriers with AO after 20 years of age. In contrast, we observed a segregation distortion unfavoring gametes carrying the expanded allele or favoring gametes carrying normal alleles. The meiotic drive towards the normal allele was more evident among female offspring. In addition, our data suggest that symptoms tend to start earlier than in former generations. If this last outcome is confirmed, the tendency for further anticipation would be associated to a reduced reproductive success in next generations. Although

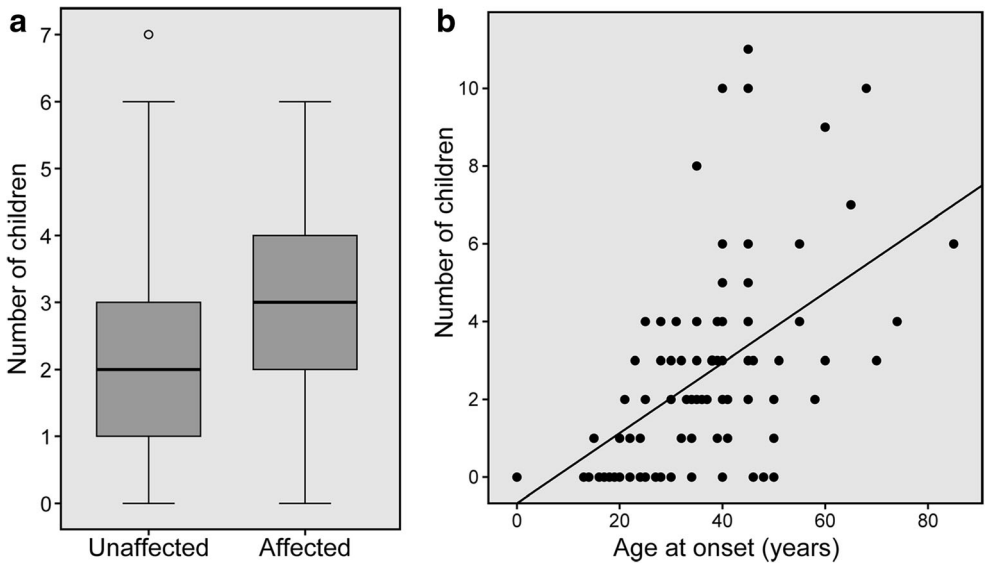


Fig. 1 Number of children among affected and unaffected individuals from SCA2 families. **a** Median number of children of affected and unaffected subjects from SCA2 families are shown in black lines in the graph ($p = 0.025$, Mann-Whitney *U* test); boxes represent interquartile distribution and error bars, upper and lower extremes. **b** Correlation between age of onset of symptoms and number of children in all symptomatic carriers included in the present study (rho = − 0.49, $p < 0.001$, Spearman)

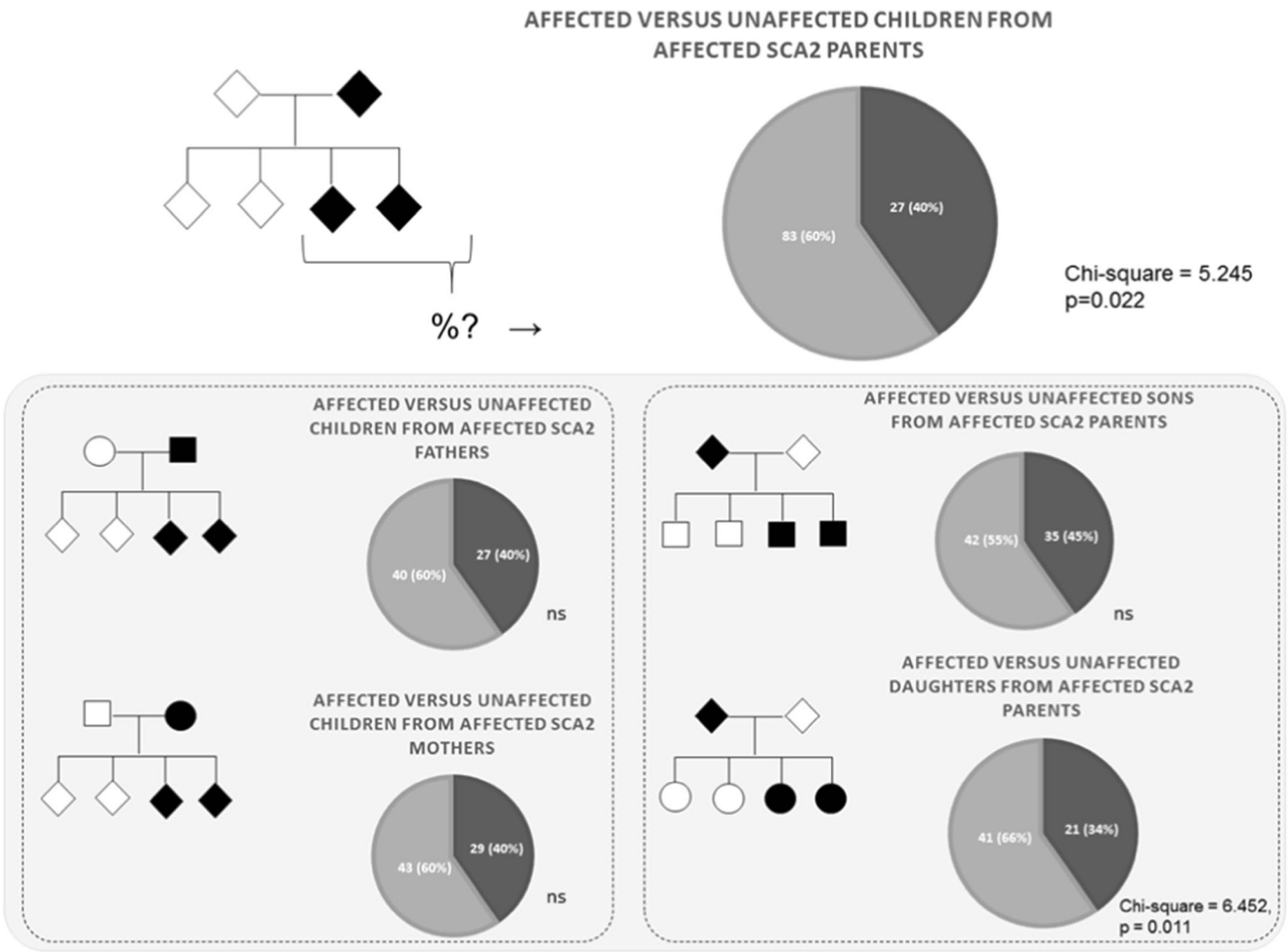
Table 1 Segregation patterns of phenotypes among offspring of symptomatic SCA2 subjects versus their unaffected sibs

			Affected sibs	Unaffected sibs	Chi-square
All informative sibs		139	56 (40.4%)	83 (59.6%)	5.245, p=0.022
Stratified according to the gender of the parent	Children of affected fathers	67	27(40.29%)	40(60.71%)	2.522, p=0.122
	Children of affected mothers	72	29(40.28%)	43(59.72%)	2.722, p=0.099
	Total	139			
Stratified according to the gender of child	Sons of affected parents	77	35(45.45%)	42(54.55%)	0.636, p=0.425
	Daughters of affected parents	62	21 (33.86%)	41 (66.14%)	6.452, p=0.011
	Total	139			

the increased fitness observed in the overall SCA2 population would contribute for an increase in frequency, the additional two forces—segregation distortion and anticipation—would work in the opposite direction, and withdraw of expanded alleles from the population would be expected.

Other polyQ diseases, such as HD, SCA1, and SCA3/MJD, have been associated to an increased reproductive success when carriers were compared to non-carriers, featuring fitnesses of 1.25, 1.47, and 1.53, respectively [14, 15]. The effect was limited to carriers with AO after 20 years of age. Since polyQ diseases tend to be related to anticipation phenomena, the increasing number of children would be limited in subsequent generations, when the transmitted CAGexp would be too large, and the associated clinical picture would be more severe. The reason why polyQ proteins like huntingtin, ataxin-1, ataxin-3, and now ataxin-2 are related to increased fertility remains unclear. Ataxin-2, for instance, is highly expressed in testes, ovaries, and other reproductive structures and tissues [21, 22]. Whatever the mechanism, the large reproductive success of polyQ diseases is a factor that contributes to keep these phenotypes/genotypes in the population.

The segregation distortion unfavoring the transmission of *ATXN2* with a CAGexp would produce a negative selection, operating against SCA2 recurrence. This outcome was in



contrast to data obtained in SCA3/MJD [16] and, indeed, might have suffered from ascertainment mistakes in the present survey, based on the phenotype after 65.7 years of age. Leaving the possibility of a type I error aside, some considerations can be drawn. Initially, distribution of normal CAG alleles at *ATXN2* and *ATXN3* are quite different in normal populations. There is large evidence showing that the *ATXN2* allele with 22 repeats is positively selected in humans [23–25]. The allele with 22 CAG repeats is so frequent that would prevent a study on *ATXN2* segregation in normal populations. Based on that, we speculate that segregation distortion, if present, would be more probably related to an advantage of the $(CAG)_{22}$ allele rather than to a disadvantage of the expanded alleles.

Anticipation is a phenomenon related to unstable transmissions of trinucleotide repeats towards further expansions [26]. Former studies raised evidence in favor of strong trends towards further increases in the number of CAG repeats when the expanded repeat is transmitted [12, 13]. However, in order to completely establish the frequency of unstable transmissions, evidence should be raised from studies that genotype all children of affected parents. These data were not available to date. If CAG expansions are really so frequent as suggested by most reports, recurrent anticipations would tend to reduce fertility towards zero.

Finally, another source of selection on polyQ diseases might be the age of the transmitting parent. Former studies were able to show that the older the male carrier of a CAGexp, the larger can be the further expansion transmitted to the offspring in SCA3/MJD [16] as well as in SCA2 [13]. We were unable to replicate this analysis due to small sample size of genotyped transmissions in our SCA2 cohort.

We are aware that our study has some limitations. On one hand, nine out of the 31 SCA2 families identified in our lab were lost and one did not agree to participate in the study. We do not know if these losses impacted on our results. On the other hand, our analyses on reproductive success and segregation were based on phenotypes due to the lack of knowledge about all *ATXN2* genotypes from members of the families under study. This issue was overcome by limiting the

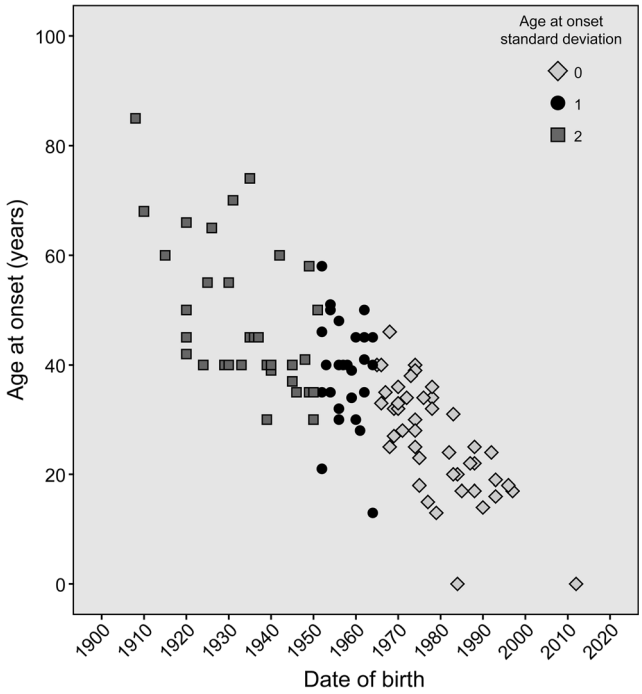


Fig. 2 Correlation between the year of birth and age of onset of symptoms among SCA2 carriers (rho = − 0.382, $p < 0.002$, Spearman). Ages at onset were characterized as older than 2, 1, or 0 standard deviations from the average age at onset

inclusion criteria to subjects older than 65.7 years of age. We are also aware that some sibs classified as unaffected could be in fact non-penetrant or very late-onset SCA2 subjects. The chance of a type II error to occur in fitness analysis—i.e., to falsely infer the absence of an increased SCA2 reproductive success due to inclusion of asymptomatic subjects among the group of unaffected subjects—was reduced, although not completely avoided. Even at risk, increased reproductive success in SCA2 was demonstrated, i.e., the present outcomes were so positive that surpassed the risk of type II error. In contrast, data on segregation distortion could still be prone to type I error, and therefore requires further clarification.

In conclusion, new evidences towards selective forces operating in SCA2 recurrence were raised by this study. Carriers of CAGexp at *ATXN2* had increased reproductive success, until the occurrence of early onset pictures, probably related to large expansions. Moreover, we suggested a potential mechanism for the selection of the $(CAG)_{22}$ allele in humans: segregation distortion. The reproductive advantage of the gamete carrying this normal allele and the anticipation phenomena associated with a decrease in the reproductive period of the affected individuals in the subsequent generation would negatively impact on the maintenance of SCA2 in populations. Finally, studies on the transmission of CAGexp are urgently needed in order to clarify the role of unstable repeats on the preservation of SCA2 in the genetic pool of humans.

Acknowledgements The authors would like to thank the individuals who agreed to participate in this study.

Funding Information This work was supported by FIPE-HCPA – Fundo de Incentivo à Pesquisa do Hospital de Clínicas de Porto Alegre – Project GPPG HCPA 16-320. EPM, GVF, MLSP and LBJ were supported by CNPq - Conselho Nacional de Desenvolvimento Científico e Tecnológico.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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