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SEQUENCE VARIATIONS IN *RAN* GENE AS POTENTIAL MODIFIERS OF AGE AT ONSET OF SPINOCEREBELLAR ATAXIA TYPE 3/MACHADO-JOSEPH DISEASE

Carolina Konrdörfer Rangel

Porto Alegre, outubro de 2020.

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Trabalho de Conclusão de Curso apresentado como requisito para obtenção de título de farmacêutico(a) pelo Curso de Farmácia da Universidade Federal do Rio Grande do Sul.

Aluna: Carolina Konrdörfer Rangel
Orientadora: Profª. Drª. Maria Luiza Saraiva Pereira

Porto Alegre, outubro de 2020.

"A gente quer passar um rio a nado, e passa; mas vai dar na outra banda é num ponto muito mais embaixo, bem diverso do em que primeiro se pensou. Viver nem não é muito perigoso?"

O Grande Sertão: Veredas
João Guimarães Rosa

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APRESENTAÇÃO

Este trabalho apresenta-se sob a forma de artigo original, que será submetido à publicação no periódico *NeuroMolecular Medicine*. As normas técnicas de instrução aos autores encontram-se disponíveis em anexo. Figuras e legendas foram colocadas no corpo do texto para facilitar a leitura dos avaliadores.

Title: Sequence variations in *RAN* gene as potential modifiers of age at onset of Spinocerebellar Ataxia Type 3/Machado-Joseph disease

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Abstract

Spinocerebellar ataxia type 3 or Machado-Joseph disease (SCA3/MJD) is the most prevalent autosomal dominant hereditary type of ataxia worldwide, causing motor incoordination by progressive neurodegeneration. The disease is due to a CAG trinucleotide expansion at the gene *ATXN3* that is inversely correlated to the disease age of onset (AO). This correlation explains about 60% of variation in AO, suggesting that genetic and/or environmental factors may act as modifiers of the disease manifestations. Neuronal intranuclear inclusions (NII) were reported to directly affect the disease progression. Genetic variations in the gene coding for Ran (ras-related nuclear protein), an essential component of the nucleocytoplasmic transport system, can interfere with NII formation and potentially modify the AO. In this study, variants rs14035 and rs7132224 were genotyped in patients of a SCA3/MJD from South Brazil. In addition, linkage disequilibrium (LD) and haplotype reconstruction were assessed, and combined haplotypes correlated to AO. No statistical differences were found between patients and control groups in allelic and genotypic distributions. However, minor allele frequencies were shown to be less represented in SCA3/MJD group for rs14035 and rs7132224 (p=0.081 and p=0.058 respectively, for genotypic distributions). The most frequent haplotype found was AC, followed by GT, corroborating with the LD found. Patients carrying GT/GT combined haplotype have, on average, a delay of 1.8 years in AO (p=0.089). Therefore, our data suggests that the studied *RAN* variants are involved in genetic modulation of AO in SCA3/MJD, enhancing the requirement for further studies evaluating the relationship between nucleocytoplasmic transport and polyglutaminopathies neurotoxicity.

Key-words: Spinocerebellar ataxia type 3, Machado-Joseph disease, *RAN* gene, Genetic modifiers, PolyQ.

1. Introduction

Spinocerebellar ataxia type 3, also known as Machado-Joseph disease (SCA3/MJD), is an inherited autosomal dominant disorder caused by a CAG expansion (CAG_{exp}) at the *ATXN3* gene, which is responsible for ataxin-3 expression (Kawaguchi et al. 1994). SCA3/MJD is the most common autosomal dominant ataxia worldwide, with a frequency of 1.5 in 100,000 inhabitants in the general population (Ruano et al. 2014). The disease leads to neurodegeneration of cerebellar, motor neuron, pyramidal and extrapyramidal areas, which comprise mainly motor and coordination function, reflecting on several movement disorders (Paulson 2012).

SCA3/MJD is a polyglutaminopathy, since CAG_{exp} leads to the expression of an abnormally long polyglutamine (polyQ) tract in the protein ataxin-3. Unaffected individuals carry 14-40 CAG repeats, while in SCA3/MJD patients stretches are expanded ranging from 51 up to more than 87 repeats (Souza et al. 2016). In general, age at onset (AO) in SCA3/MJD is inversely correlated to CAG_{exp}, but the causal mutation explains just 50-65% of the interpatient variability in AO (Kuiper et al. 2017). Hence, disease modifiers, such as genetic and/or environmental factors, might change the clinical history of patients, perhaps by modulating AO and other phenotypes, such as severity of symptoms and velocity of disease progression. Some genetic and molecular modifiers of AO have been described for SCA3/MJD, such as single nucleotide polymorphisms (SNPs) in genes including *GRIK2*, *IL1B*, *NEDD8*, and *NEDD9* (Emmel et al. 2010), *APOE* (Bettencourt and Lima 2011), *FAN1* (Mergener et al. 2020), CAG repeat length of the non-expanded *ATXN3* allele (Tezenas du Montcel et al. 2014; Chen et al. 2016), length of CAG repeats at the *ATXN2* gene (du Montcel et al. 2014), degree of methylation of the *ATXN3* promoter (Emmel et al. 2011) and HSP40 expression levels (Zijlstra et al. 2010). However, most of these findings were not replicated by other studies. Furthermore, none of these genetic modifiers resulted in treatment possibilities so far.

PolyQ expanded stretches facilitate the aggregation and intraneuronal accumulation of ataxin-3. Accordingly, the biological hallmark of SCA3/MJD is the presence of polyQ neuronal intranuclear inclusions (NII) (Nóbrega et al. 2018). Although the complete mechanism of SCA3/MJD has not been fully elucidated, the presence of polyQ NII is seen as a harmful event, related to gain of toxic function by mutant ataxin-3, which is required for disease-related neurodegeneration (Bichelmeier et al. 2007; Ramani et al. 2017; Sowa et al. 2018). Nevertheless, NII have also been described as less toxic than cytoplasmic inclusions in neurodegeneration (Seidel et al., 2017). Albeit there are dissonant views on the effect of NII in the onset and/or progression of in neurodegenerative diseases (Arrasate et al. 2004; Woerner et al. 2016), modulation of aggregate formation and cellular localization might be critical in SCA3/MJD and other polyQ diseases.

Mutant ataxin-3 is subjected to caspase and calpain cleavage into peptides as a cellular protective attempt, although this process is directly associated with disease severity (Hübener et al. 2013). The Ras-Related Nuclear Protein (Ran) is a GTP-binding nuclear protein essential for the import and export of different molecules through the nuclear pore complex (NPC) (Sazer and Dasso 2000; Macara 2001). Although molecules with less than 40 kDa can freely pass through the NPC via passive transport (Grima et al. 2017), larger peptides depend on active transport by Ran gradient between the nucleus and the cytoplasm. In cellular, mouse and *Drosophila* models, both full-length and truncated mutant ataxin-3 are transported to the nucleus via nuclear localization signals (NLS) in a Ran-GTPase dependent manner (Sowa et al. 2018; Wang et al. 2019). Therefore, considering Ran-mediated nucleocytoplasmic shuttling of mutant ataxin-3 and the straight relationship between

NII and neurodegeneration, it is tempting to speculate that biological differences in this system might contribute to AO variability in SCA3/MJD. Specifically, variable levels/activity of Ran due to rare genetic variants might predispose neurons to accumulate variable cytoplasmic ataxin-3 aggregates, thus hastening or retarding the disease onset.

In this work, we aimed to investigate the potential influence of genetic variability in *RAN* on age at onset of SCA3/MJD. We addressed whether *RAN* polymorphisms rs14035 and rs7132224 correlate with AO in a South Brazilian group of SCA3/MJD patients. Furthermore, haplotype reconstruction and linkage disequilibrium between variants were also assessed.

2. Materials and Methods

Patients and samples

Two hundred and eight SCA3/MJD patients from the Rio Grande do Sul, Brazil, were investigated in this study. DNA samples from study participants were obtained from the Serviço de Genética Médica (SGM) biorepository and were analysed at the Translational Neurogenetics Laboratory, both from Hospital de Clínicas de Porto Alegre (HCPA). As inclusion criteria, individuals had to be clinically symptomatic, with a confirmed molecular diagnosis of SCA3/MJD and known AO. AO was defined as the age when the patient or a close relative first noticed any symptoms (usually gait ataxia). The anonymized use of DNA samples from these patients for research purposes only was assessed and approved by the HCPA institutional ethics committee. Eighty-three unrelated healthy individuals from the same region were analysed as controls, in order to establish allelic and genotypic frequencies of the selected SNPs among the local population. Expected AO were estimated by generalized linear models described by de Mattos et al. (2019) specific for patients from the Rio Grande do Sul biorepository, taking into account the CAG_{exp} length of each patient included in this study.

DNA isolation and determination of *ATXN3* CAG length

DNA samples were obtained from peripheral blood leukocyte fraction as previously described by standard protocol. DNA concentration was quantified using a NanoDrop device (ThermoFisher) and samples were diluted to either 2 ng/µl for SNPs genotyping or 50 ng/µl for determining CAG length. Length of CAG tract of both *ATXN3* alleles was evaluated by PCR amplification with fluorescently-labelled primers flanking the CAG repeat-containing region in exon 10. Amplification products were then mixed with formamide (HiDye formamide, Applied Biosystems) and GeneScanTM 500 LIZ (Applied Biosystems), and analysed by capillary electrophoresis in an ABI3130*xl* Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Amplicon lengths were then determined by GeneMapper® ID v 3.2 software (Applied Biosystems, Foster City, CA, USA), as described previously (França et al. 2012).

RAN polymorphisms and genotyping

The selection of *RAN* SNPs was based on the following criteria: minor allele frequency (MAF) higher than 0.10; gene location (preferably in exons and/or regulatory regions); clinical significance; influence on gene expression. As filters, the platforms Ensembl Genome Browser, GTEX portal and VarSome were accessed. Based on that, rs14035 and rs7132224 were chosen to be evaluated in this study.

rs14035 and rs7132224 were analysed by Taqman® SNP Genotyping Assay (ThermoFisher) with probes C_11351340_10 and C_189223196_10, respectively. Reactions were performed in a final volume of 8 µl, containing 4 ng DNA, 0.2 µl Taqman® probe and 4 µl 2X PCR Genotyping Master Mix, according to the assay protocol (Applied Biosystems, Foster City, CA, USA). For amplification, an initial step of 2 minutes at 50ºC was performed to guarantee uracil-N-glycosylase (UNG) activation, followed by AmpliTaq Gold™ activation at 95ºC for 10 minutes and 40 cycles of denaturation at 95ºC for 15 seconds followed by annealing

and extension at 60ºC for 1 minute. Amplification and analysis of PCR products were performed in an ABI Prism 7500 Fast Real-Time PCR System® equipment (Applied Biosystems, Foster City, CA, USA).

In silico analysis

Reconstruction of *RAN* haplotypes from SCA3/MJD and control groups was performed using PHASE software version 2.1.1. Linkage disequilibrium (LD) for the predicted haplotypes data was analysed by Arlequin version 3.5.2.2, considering the LD coefficient (D') and r^2 (which also takes into account allele frequency) as factors to define strong or weak LD. Allelic and genotypic frequencies were compared between data from the present study and different populations using publicly available genetic databases from the 1000 Genomes Project (Zerbino et al. 2018) and GnomAD (Lek et al. 2016).

Statistical analysis

The Predictive Analytics SoftWare (PASW) Statistics program 18.0 (SPSS Inc., Chicago, IL, USA) was used to perform all statistical analysis. Allelic and genotypic frequencies between SCA3/MJD and control groups as well as Hardy-Weinberg equilibrium (HWE) were analysed by the Pearson Chi-Square test. In order to test the influence of *RAN* variants on AO, mean residual age at onset (RAO), defined as the difference between the observed and expected AOs, was compared between genotypes (using both co-dominant and recessive models) and combined haplotypes using one-way analysis of variance (ANOVA) or Student's t test. Kolmogorov-Smirnov test was used to test conformity of RAO to a normal distribution. Levene's test and residual diagnostics were used to check sample homoscedasticity for ANOVA and regression tests, respectively. Both the degree of variability in AO explained by CAG_{exp} as well as the potential improvement in explanation afforded by inclusion of *RAN* genotypes were predicted by generalized linear models, and reported as changes in the R^2 coefficient. ANOVA, Student's test and regression analyses considered a mixed model to avoid bias regarding the inclusion of more than one SCA3/MJD individual from the same family (Sainani 2010). Results were considered statistically significant when $p<0.05$.

3. Results

Among 208 SCA3/MJD patients samples (56% females), CAG_{exp} length ranged from 68 to 84 (mean ± SD: 75.3 ± 3.3), while CAG length of the non-expanded *ATXN3* alleles ranged from 13 to 37 repeats (mean ± SD: 22.1 ± 4.7). In 141 cases, more than one patient from the same family was included in the study (mean: 1.8 individuals per family; range 1 to 8 individuals; 112 families) (Table 1). To avoid family bias, a statistical mixed model was adopted in our analysis. In the present sample group, CAG_{exp} alone explained about 62% of the variability in AO (r=-0.791, R^2=0.620, p<0.001).

Table 1 Sample characterization

Sample features	Total (n=208)
Women	117 (56.2%)
AO (years)	34.32 (10-56)
AO predicted at birth (years)	35.07 (12-53)
Patients per family	1.86 (1-8)
Normal allele $(CAG)_n$	22.13 (13-37)
Expanded allele $(CAG)_{exp}$	75.32 (68-84)

AO stands for age of onset. Data are given as n (absolute value) and mean (range from minimum to maximum value)

Two SNPs in the *RAN* gene were selected for genotyping analyses using the criteria described above. rs14035 is located at 3' UTR region, while rs7132224 is located at 5' UTR region, at a transcription factor (TF) binding site. Allelic and genotypic frequencies from both rs14035 and rs7132224 were in Hardy-Weinberg equilibrium for SCA3 (p=0.171; p=0.086, respectively) and control groups (p=0.163; p=0.258, respectively). Global MAFs for both variants were closer to the patients' group distribution than to the control group (Table 2).

Table 2 Variants information and HWE

Variant	MAF	Frequency of variant		HWE		Chromosomal Location	Gene Location
		SCA3/ MJD	***Local controls***	***SCA3/ MJD***	***Local controls***		
rs14035	0.271	0.281	0.339	p=0.171	p=0.163	12:130876696	3' UTR variant
rs7132224	0.303	0.286	0.357	p=0.086	p=0.258	12:130871501	5' UTR variant

MAF global minor allele frequency. HWE Hardy-Weinberg Equilibrium. Location according to Ensembl. Data are given in frequency

No difference was found between SCA3/MJD and control groups when comparing allelic and genotypic frequencies for rs14035 (p=0.165; p=0.081, respectively) and rs7132224 (p=0.092; p=0.058, respectively), although data suggests higher frequency of heterozygotes in the control group (Table 3).

Table 3 Allelic and genotypic frequencies found in MJD/SCA3 and local groups

		SCA3/MJD n=208	*Local controls n=84*	*p value*
rs14035				
Alleles	C	299 (0.719)	111 (0.661)	0.165
	T	117 (0.281)	57 (0.339)	
Genotypes	CC	111 (0.534)	34 (0.405)	0.081
	CT	77 (0.370)	43 (0.512)	
	TT	20 (0.096)	7 (0.083)	
rs7132224				
Alleles	A	297 (0.714)	108 (0.643)	0.092
	G	119 (0.286)	60 (0.357)	
Genotypes	AA	112 (0.538)	33 (0.393)	0.058
	AG	74 (0.356)	42 (0.500)	
	GG	22 (0.106)	9 (0.107)	

Data are given as n (absolute value) and frequency. Statistical analysis using Pearson Chi-square test. $p< 0.05$

In addition, allelic and genotypic frequencies of both SNPs from patient and control groups were compared to frequencies among different populations from the GnomAD and 1000 Genomes project databases. For both variants, while the SCA3/MJD group had allelic and genotypic frequencies closer to American and East Asian populations, frequencies in the control group were more similar to those of European populations (Fig. 1).

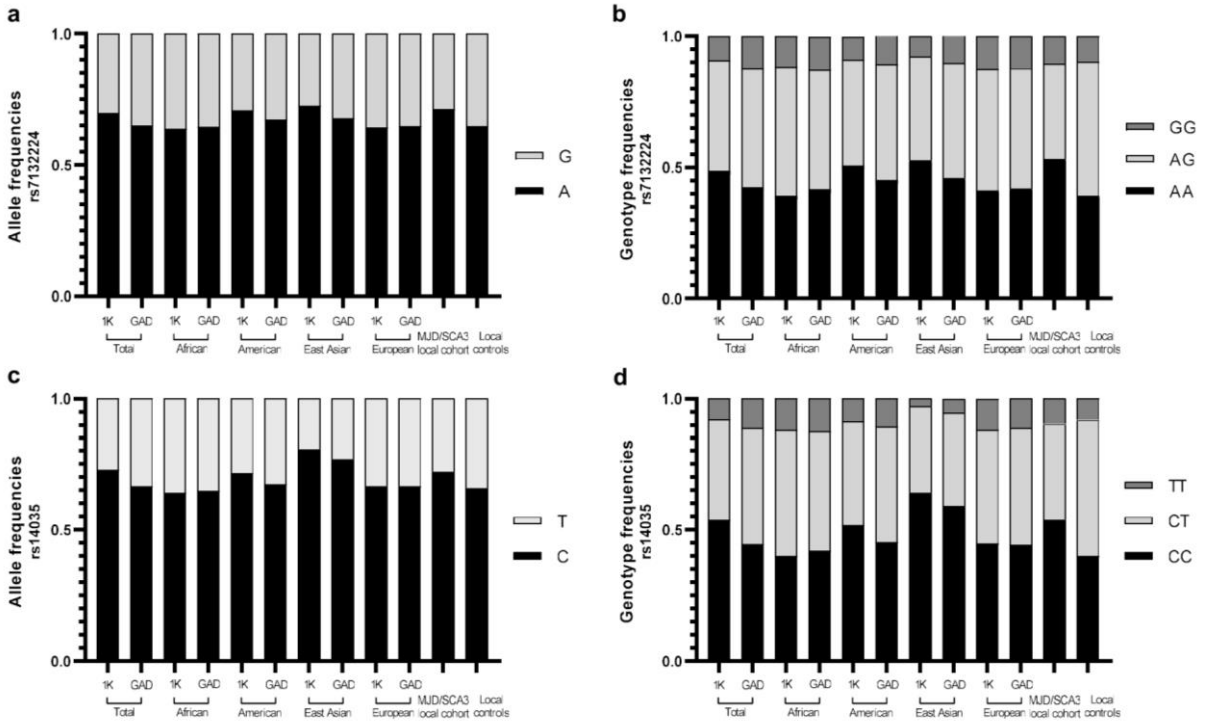

Fig. 1 Allele and genotype frequencies of *RAN* single nucleotide polymorphisms rs7132224 and rs14035 in different ethnic groups. **a** Allele frequencies for rs7132224. **b** Genotype frequencies for rs7132224. **c** Allele frequencies for rs14035. **d** Genotype frequencies for rs14035. Total: average frequency in all populations combined. 1K stands for 1000Gnomes and GAD stands for GnomAD

Haplotype reconstruction for variants rs14035 and rs713222 was performed by PHASE and the resulting distribution is shown in Table 4. LD was calculated using the predicted haplotypes. Both SCA3/MJD and control groups presented LD coefficient (D') and r^2 closer or equal to 1.0 (Table 4). Therefore, the two variants are in strong LD.

Table 4 Variants' haplotypes distribution and linkage disequilibrium

		SCA3/MJD n=208			*Local controls n = 84*		
Variant	Haplotype	n	r^2	D'	n	r^2	D'
rs14035 rs7132224	GC	5 (0.012)	0.9077	|0.9641|	3 (0.018)	0.9243	|0.1000|
	GT	114 (0.274)			57 (0.339)		
	AC	294 (0.707)			108 (0.643)		
	AT	3 (0.007)			0		

Data are given as n (absolute value) and frequency. r2 and D' are coefficients of linkage disequilibrium. Values range from 0 to 1, where values closer to 1 indicate LD association.

We then combined the different haplotypes found in the same patient to investigate the effect of both variants on AO. Strangely, the most prevalent combined haplotypes were AC/AC for the SCA3/MJD group and AC/GT for the control group (Table 5).

Table 5 Distribution of combined haplotypes

Combined Haplotype	*SCA3/MJD* *n = 208*	*Local controls* *n = 84*	*p* value
AC/AC	108 (0.519)	33 (0.393)	0.172
GT/GT	20 (0.096)	7 (0.083)	
AC/GT	72 (0.334)	41 (0.488)	
AC/GC	3 (0.014)	1 (0.012)	
AC/AT	3 (0.014)	0 (0.000)	
GC/GT	2 (0.010)	2 (0.024)	

Data are given as n (absolute value) and frequency. Statistical analysis using Fisher's Exact Test. $p < 0.05$

Since both variants rs14035 and rs7132224 are in high LD, the investigation regarding their influence in SCA3/MJD AO was performed using reconstructed haplotypes. Based on the hypothesis that homozygous individuals for rare variants (GT/GT) might show variability of the expected AO, we compared this combined haplotype (GT/GT) against the other 5 combined haplotypes found (non-GT/GT group) through mixed model and linear regression. A high inverse correlation between CAG_{exp} repeat length at *ATXN3* and AO was found (adjusted $R^2 = 0.620$; $p < 0.001$). For the non-GT/GT group the inverse correlation showed an $R^2 = 0.621$, while GT/GT group had $R^2 = 0.769$, enhancing the AO variability explication (Fig. 2).

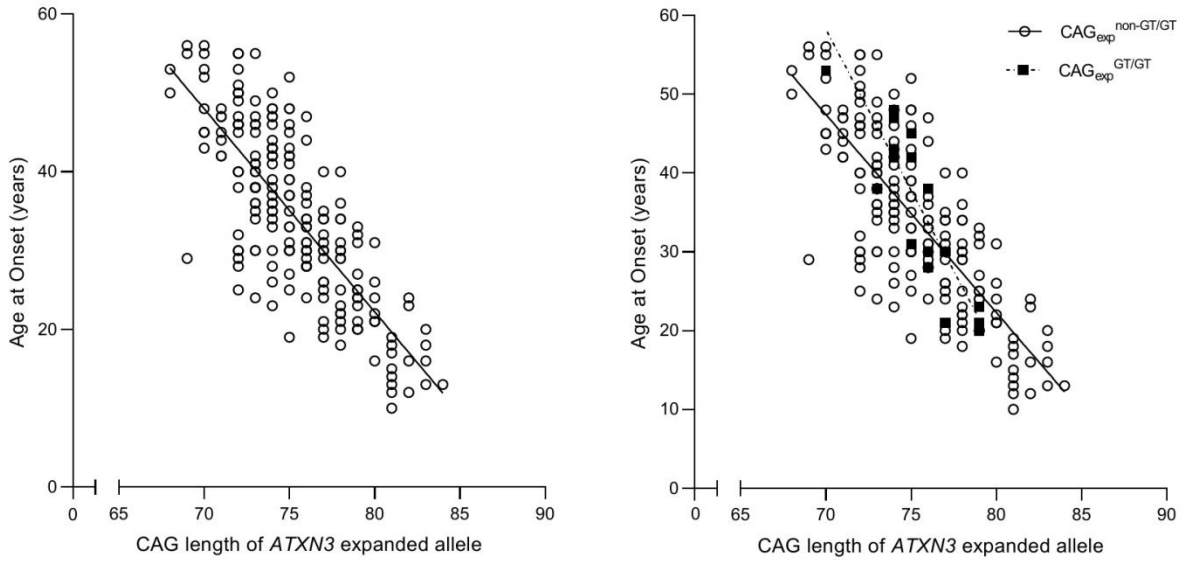

Fig. 2 Correlation between age of onset (AO) and CAG_{exp} repeat length at *ATXN3*. **a** Correlation between age of onset (AO) and CAG_{exp} repeat length at *ATXN3*. Circles represent AO of subjects (n=208). Line represents the linear regression model of AO (R^2=0.620). **b** Linear regression between AO and CAG_{exp} considering the minor allele of rs14035 and rs7132224 in

homozygosis (combined haplotype GT/GT). (■) represents GT/GT combined haplotype individuals. (○) represents non-GT/GT group. Dashed line represents the linear regression model of AO for GT/GT combined haplotype (n = 20; R^2 = 0.769). Filled line represents the linear regression model of AO for the non-GT/GT group (n = 188; R^2 = 0.621)

When looking at RAO, patients presenting the combined haplotype GT/GT have, on average, the AO potentially delayed by 1.8 years from the AO expected, while patients within non-GT/GT group presented, on average, an advancing on AO by 0.9 years ($p = 0.089$), suggesting that GT/GT combined haplotype is a potentially protective SCA3/MJD genetic modifier (Table 6).

Table 6 Influence of GT/GT combined haplotype on RAO of SCA3/MJD patients

Combined Haplotype$^{rs7132224/rs14035}$	Mean RAO	*s*	*p* value
$RAN^{non\text{-}GT/GT}$ *n= 188*	-0.88 (-22.13 to -9.79)	6.726	0.089
$RAN^{GT/GT}$ *n= 20*	1.85 (-9.78 to 9.58)	5.980	

RAO stands for residual age at onset, defined as the difference between the AO observed and the AO expected; *s* Standard deviation. Values obtained through statistical mixed model. $p < 0.05$

4. Discussion

In this work genetic variants rs14035 and rs7132224 were genotyped in a group of SCA3/MJD patients and influence of these SNPs on AO was assessed. We have also described the frequency of these variants in a local control group, which is the first reported Brazilian sample. About 62% of the cases have the AO explained by the CAG expanded length in our sample, percentage among the range of values already described for this biorepository (Saute and Jardim 2015; Mergener et al. 2020). When comparing variants distribution in patients and control groups, there were no statistical differences on allelic or genotypic frequencies ($0.10>p>0.05$). Nevertheless, whilst SCA3/MJD group showed a higher genotypic frequency for the wild allele in homozygosis, control group presented in majority a heterozygous pattern (Table 3).

According to the 1000 Genomes Project and GnomAD databases, looking at the both variants population distribution, the minor allele T at rs14035 has a frequency estimated in 33% in Europeans, 35-36% in Africans, 19-23% in East Asians, and 28-32% in Americans. This same variant has shown a frequency distribution of 28% for SCA3/MJD, which is similar to the frequency described in Americans, while the control group frequency was estimated to be 34%, placing between Europeans and Africans. The frequency of the minor allele G at rs7132224 was estimated to be 35-36% in Europeans, 35-36% in Africans, 27-32% in East Asian, and 29-33% in Americans. Frequency of this allele in SCA3/MJD patients was estimated in 28%, being between East Asians and Americans, differently from the control group (Fig. 1). The finding on similarity of the control group to the European population for both variants might be related to the fact that more than 80% of the population from South Brazil is descended from Europeans (Ruiz-Linares et al., 2014). However, the similarity of SCA3/MJD group distributions to American and East Asian populations cannot be addressed by founding effect, once there are no evidences on migration of these population groups to south Brazil. Hence, our data suggests that the inferred different allelic distribution for the studied variants between SCA3/MJD and control groups might be related to the CAG expansion at the *ATXN3* gene. Therefore, further studies are required.

PolyQ neuronal intranuclear inclusions (NII) are the SCA3/MJD hallmark, and its nuclear localization has been considered a neurotoxic factor, which modulates the disease progression (Nóbrega et al. 2018). Nevertheless, the complete mechanism on how PolyQ neuronal inclusions might turn into NII remains unclear. Although research groups have shown some nucleocytoplasmic system components relation with poliglutaminopathies (Grima et al. 2017; Sowa et al. 2018), related genetic factors were still not completely explored. Hence, in this work we addressed the effect of two variants in *RAN* on AO of SCA3/MJD patients, in order to understand a possible influence of Ran-GTPase system on the disease onset. Following genotyping assays, LD was found between variants in both patients and control groups, corroborating with other population research findings (Yates et al. 2020). We then performed haplotype reconstruction in order to analyse both variants distribution and investigated the influence of combined haplotypes on SCA3/MJD AO, taking into account CAG_{exp}. Remarkably, while CAG_{exp} explains 62% of the cases' AO, considering the potential effect of GT/GT combined haplotype, the percentage increases to 76%. Moreover, our results suggest GT/GT combined haplotype as a protective modifier of the disease onset, with a delay of 1.8 years. Given that only 20 individuals of our sample group had this genetic profile, further survey with larger sample size is required for conclusive data (Table 5). Additionally, taking into account the disease frequency, the effect of this rare combined haplotype in SCA3/MJD patients cannot be ruled out.

Genetic modifications can interfere in protein function in many ways. rs14035 is located in the regulatory 3' UTR region of *RAN* and has being studied as a miRNA biogenesis variant (Horikawa et al. 2008; Mullany et al. 2016). Some rs14035 genotypes have been related to vascular complications (Kim et al. 2018; Ko et al. 2019; Wen et al. 2019), recurrent pregnancy loss risk (Jung et al. 2014), and cancer (Li et al. 2020). On the other hand, variant rs7132224 is located in a TF binding site, and had been related to neuroblastoma (J. Wang et al. 2018) and Wilms tumor (Huang et al. 2020), despite studies regarding the variant remain scarce. Although variants are located in different gene regions, both are associated with variability of Ran expression. While rs14035 is located in a miRNAs binding region and, therefore, may cause changes in gene suppression (Esquela-Kerscher and Slack 2006), variant rs7132224 is located in an essential gene expression regulation site, modulating TF bindings. In cases of neurodegenerative diseases such as SCA3/MJD, the presence of homeostasis disruption (Da Silva et al. 2019) and increased demand on nucleocytoplasmic transport (Seidel et al. 2017) might turn slight variations in Ran expression determinant on the disease progression by variability of NII formation.

Despite Ran relation to nucleocytoplasmic transport, the protein is also an androgen receptor (AR) coactivator, known as ARA24 (Bischoff and Ponstingl 1991). Interestingly, ARA24 interaction with PolyQ chains within AR is variable, lying on the extension of PolyQ length (Hsiao et al. 1999). Therefore, Ran relation to CAG_{exp} within the cell in polyglutaminopathies might also vary. Moreover, different frequency on NII presence relying on huntingtin CAG tract length was demonstrated previously, evidencing a relation between nucleocytoplasmic transport machinery and PolyQ length (Martindale et al. 1998).

Remarkably, there are still no effective treatments to prevent or to delay neurodegeneration onset in SCA3/MJD (Da Silva et al. 2019). Although the increasing data regarding SCA3 pathophysiology, few is known about NII influence in disease severity and, accordingly, no related molecule has been targeted as a therapeutic compound to date. The complete cellular mechanisms and the key factors that lead to neurodegeneration in SCA3/MJD need to be elucidated in order to offer novel and effective treatment approaches.

In summary, we have firstly described rs14035 and rs7132224 allelic and genotypic frequencies in a SCA3/MJD group as well as shown LD between variants in this specific population through haplotype reconstruction. Our data suggests different variant genotypic distributions among patients and control groups, which require further investigation for enlightenment. Finally, a delay of 1.8 years in AO was observed in SCA3/MJD patients carrying the combined haplotype GT/GT, suggesting a protective effect. Therefore, our data strengths the hypothesis that nucleocytoplasmic transport processes might affect the onset of SCA3/MJD, suggesting that variants in *RAN* might play a role as genetic modifiers in this disease.

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Declarations

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

Ethical Approval: The study was performed as per the revised Helsinki declaration following approval of the ethics committee of the hospital from where samples were collected.

Informed Consent: Informed consent was obtained from all individual participants included in the study.

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ANEXO 1 – REGRAS DA REVISTA

Instructions for Authors

Article Types

Original Articles: Full-length reports of current research. Abstract: 250 words maximum. Article: 6,000 words including abstract and acknowledgement but excluding author contributions statement, disclosure, references, figure legends tables and figures (Up to 8 in total figures + tables).

Review Articles: Reviews are comprehensive analyses of specific topics relevant to mechanistic understanding or therapeutic development of a CNS condition. **Abstract**: 250 words maximum Article: 10,000 words including abstract, figure legends and acknowledgements. A maximum of 150 references are permitted.

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Vascular Dementia
Adult Neurogenesis
Exercise-related Metabolism
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References

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- This result was later contradicted by Becker and Seligman (1996).
- This effect has been widely studied (Abbott 1991; Barakat et al. 1995; Kelso and Smith 1998; Medvec et al. 1999).

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The list of references should only include works that are cited in the text and that have been published or accepted for publication. Personal communications and unpublished works should only be mentioned in the text. Do not use footnotes or endnotes as a substitute for a reference list.
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- Article by DOI Slifka, M. K., & Whitton, J. L. (2000) Clinical implications of dysregulated cytokine production. *Journal of Molecular Medicine*, https://doi.org/10.1007/s001090000086
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- Online document Abou-Allaban, Y., Dell, M. L., Greenberg, W., Lomax, J., Peteet, J., Torres, M., & Cowell, V. (2006). Religious/spiritual commitments and psychiatric practice. Resource document. American Psychiatric Association. http://www.psych.org/edu/other_res/lib_archives/archives/200604.pdf. Accessed 25 June 2007.

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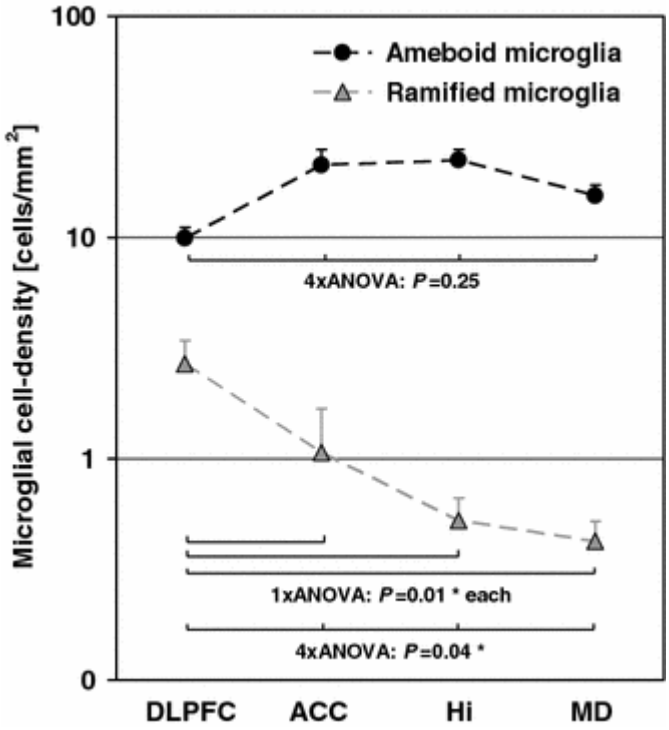

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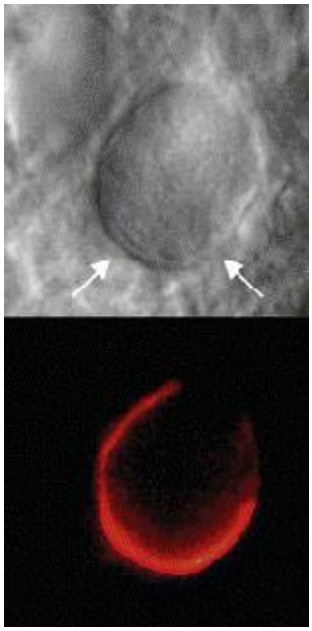

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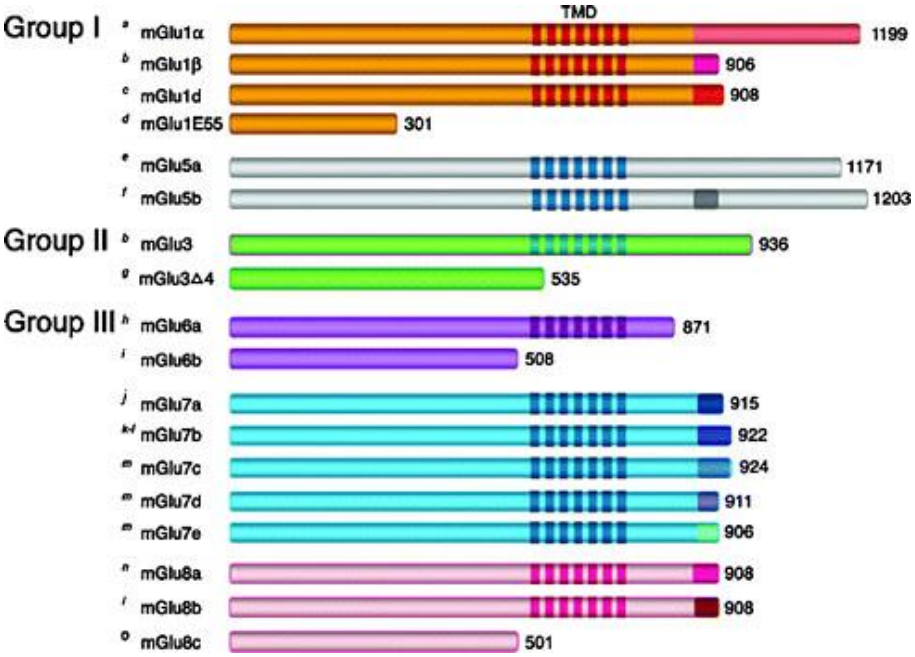

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