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RESEARCH

Machado Joseph-Disease Is Rare in the Peruvian Population

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Abstract

Spinocerebellar ataxia type 3 or Machado-Joseph disease (MJD/SCA3) is the most prevalent autosomal dominant cerebellar ataxia worldwide, but its frequency varies by geographic region. We describe MJD/SCA3 patients diagnosed in a tertiary healthcare institution in Peru. In a cohort of 341 individuals (253 probands) with clinical ataxia diagnosis, seven MJD/SCA3 probands were identified and their pedigrees extended, detecting a total of 18 MJD/SCA3 cases. Out of 506 alleles from all probands from this cohort, the 23-CAG repeat was the most common *ATXN3* allele (31.8%), followed by the 14-CAG repeat allele (26.1%). Normal alleles ranged from 12 to 38 repeats while pathogenic alleles ranged from 64 to 75 repeats. We identified 80 large normal (LN) alleles (15.8%). Five out of seven families declared an affected family member traced back to foreign countries (England, Japan, China, and Trinidad and Tobago). MJD/SCA3 patients showed ataxia, accompanied by pyramidal signs, dysarthria, and dysphagia as well as abnormal oculomotor movements. In conclusion, *ATXN3* allelic distribution in non-MJD/SCA3 patients with ataxia is similar to the distribution in normal individuals around the world, whereas LN allele frequency reinforces no correlation with the frequency of MJD/SCA3. Evidence of any atypical MJD/SCA3 phenotype was not found. Furthermore, haplotypes are required to confirm the foreign origin of MJD/SCA3 in the Peruvian population.

Keywords Allelic distribution · *ATXN3* · Machado-Joseph disease · Peruvian ataxic patients · MJD/SCA3 phenotype · Spinocerebellar ataxia type 3

Introduction

Spinocerebellar ataxia type 3 or Machado-Joseph disease (MJD/SCA3) is a late-onset autosomal dominant neurodegenerative disease associated with abnormal expansion of the CAG microsatellite within the *ATXN3* gene (14q32.1) [1]. MJD/SCA3 shows marked phenotypic variability: from pure ataxic forms associated with other signs such as neuropathies or parkinsonism, to forms with severe spasticity and dystonia [2]. The symptoms have traditionally been classified into differential clinical types or subphenotypes including cerebellar ataxia, pyramidal signs, extrapyramidal signs—dystonia and parkinsonism—peripheral neuropathy, spastic paraplegia, and ophthalmoplegia [3, 4]. Non-motor symptoms such as autonomic dysfunction, fatigue, pain, neuropsychiatric symptoms, and sleep disorders have also been described [5].

MJD/SCA3 is considered the most common dominant inherited ataxia worldwide with a variable frequency

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depending on the geographic region [6]. The relative frequency of MJD/SCA3 among dominant ataxias is highest in Brazil (59.6%) [7] and Portugal (57.8%) [8], whereas countries such as Italy (1.1%) [9] and South Africa (3.7%) [10] have reported lower relative frequencies. In Latin America, it is well known that Brazil has the highest frequency of MJD/SCA3, although information about other countries is still limited. Venezuela also has a considerable relative frequency of 25% [11], followed by Chile (20%) [12]; Argentina (20%) [13]; Mexico (12%) [14]; and Cuba (1.2%) [15].

In Peru, there are anecdotal reports on MJD/SCA3 cases [16], suggesting a low frequency of this type of ataxia in this country. The main purposes of this study are to analyze the frequency of MJD/SCA3 within ataxic patients in Peru, and to describe phenotype and *ATXN3* allelic distribution within the Peruvian MJD/SCA3 population.

Materials and Methods

Study Design and Population

We conducted a cross-sectional study to describe the clinical and molecular characteristics of MJD/SCA3 patients with extended pedigrees, as well as to explore the allelic distribution of *ATXN3*-[$CAG_{(n)}$] within a cohort of ataxic patients screened for MJD/SCA3 at a neurogenetics outpatient clinic in Lima, Peru. From January 1996 to February 2022, a total of 341 individuals from both genders with clinical diagnosis of hereditary ataxia followed up at a tertiary hospital were included in this study. The exclusion criteria were (a) the presence of any abnormality on the following tests at recruitment that explained the occurrence of ataxia or related neurological symptoms: basic blood biochemistry; vitamin B12, vitamin E, VDRL (venereal disease research laboratory); chest X-ray; abdominal ultrasound; mammary ultrasound and mammography (in women); thyroid-stimulating hormone, lymphocyte and thrombocyte count, hemoglobin, erythrocyte mean corpuscular volume, sedimentation rate, antibodies (anti-HIV, human immunodeficiency virus), qualitative urine test, anti-Yo, and anti-Hu antibodies; (b) suggestive MRI for a vascular, autoimmune, or infectious process in the central nervous system. Informed consent was obtained from each participant. This study was approved by the Institutional Review Board from the *Instituto Nacional de Ciencias Neurológicas*, Lima, Peru.

ATXN3 Genotyping

Blood samples were collected, then DNA was isolated from leukocytes at the Neurogenetics Lab in Lima using standard procedures [17]. DNA samples underwent *ATXN3* genotyping based on the amplification of the CAG repeat within *ATXN3* by polymerase chain reaction (PCR) employing a modified procedure originally standardized by Kawaguchi et al. [18]. The modified PCR protocol had the following conditions: 1X PCR buffer, 10% DMSO, 1.5 mM MgCl2, 0.15 mM of each dNTPs, 1 µM of each primer (MJD52 5′-CCAGTGACTACTTTGATTCG-3′ and MJD25 5′- TGG CCTTTCACATGGATGTGAA-3′), 0.5 U of Platinum Taq DNA Polymerase, and 0.3 ng of DNA genomic in a final reaction volume of 10 µL. The amplification program had the following initial conditions: 2 min of initial denaturation at 94 °C; 28 cycles of 30 s denaturation at 94 °C, 30 s hybridization at 58.5 °C, and 1 min extension at 72 °C; and 10 min of final extension at 72 °C. The amplicons were observed by 6% non-denaturing polyacrylamide gel electrophoresis. Allele sizing was performed using reference samples of known genotype analyzed by PCR and capillary electrophoresis through Rede Neurogenetica-Brazil.

Samples not displaying both alleles were further genotyped by triple repeat-PCR (TP-PCR) followed by 10% non-denaturing polyacrylamide gel electrophoresis. The TP-PCR was performed employing a modified procedure originally standardized by Melo et al. [19]. The modified TP-PCR protocol had the following conditions: 1X PCR buffer, 6% DMSO, 1.5 mM MgCl2, 0.2 mM of each dNTPs, 0.3 µM of primer MJD25R (5′-TGGCCTTTCACATGGATGTGA-3′), 0.06 µM of primer ForIntRep (5′-TACGCATCCCAGTTT GAGACG-3′), and 0.3 µM of primer ForTail (5′-TACGCA TCCCAGTTTGAGACGCAGCAGCAGCAGCAG-3′); 0.4 U of Platinum Taq DNA Polymerase and 10 ng of DNA genomic in a final reaction volume of 10 µL. The amplification program had the following initial conditions: 2 min of initial denaturation at 94 °C; 30 cycles of 30-s denaturation at 94 °C, 30-s hybridization at 62 °C, and 45-s extension at 72 °C; and 10 min of final extension at 72 °C.

Due to the lack of consensus to date, we classified *ATXN3* alleles according to Saute and Jardim et al. [20]. Twelve to 44 CAG repeat length alleles are considered normal and ≥ 51 CAG repeats as pathogenic. Within normal alleles, we identified a subgroup of large normal (LN) alleles with ≥ 27 CAG repeats [21].

MJD/SCA3 Extended Families Identification and Data Collection

Among the 341 participants with ataxic symptoms, we identified seven non-related MJD/SCA3 probands. We extended the pedigrees of all seven probands and contacted most of the affected family members. We performed home visits to a total of 18 affected individuals by home visits in four different cities across the country to complete a standardized clinical questionnaire, neurological examination, SARA, MoCA, and PHQ-9 rating scales assessment. Extended pedigrees were drawn using an online pedigree tool (https://www.

progenygenetics.com). Age at onset was defined when the patient or a close relative noticed the first symptom of the disease, which was usually but not always gait ataxia. We collected information on the presence or absence of several neurological findings, as well as clinical and family history.

Statistical Analysis

We estimated *ATNX3* allelic frequencies and presented their distributions in vertical bars, grouped according to classification mentioned above. We calculated the mean, median, and mode of the allelic frequency.

Results

Identified MJD/SCA3 Pedigrees

We found 18 MJD/SCA3-affected individuals (six women) from seven different families.

The mean age at sample drawing/recruitment was 59.7 ± 12.6 years (range 40 to 77 years). Expanded pedigrees showed that five out of seven SCA3/MJD3 families declare a foreign origin (Fig. 1). The age at onset ranged from 20 to 64 years, with an average of 46.3 ± 13.3 years. Age at onset was inversely correlated with the ATXN3-(CAG)n in the expanded allele ($r = -0.55$, $p = 0.01$). The mean duration of disease was 14.3 ± 10.6 years and the mean time at definitive diagnosis was 12.8 ± 10.8 years. There was no predominance regarding paternal transmission (50%). The mean SARA score was 12.2 ± 7.1 ($n = 16$) and mean NESSCA score was 11.8 ± 4.9 ($n = 15$). Exploratory analysis showed that high SARA score was positively correlated with disease duration ($r = 0.64$, $p = 0.01$). We did not find significant association with NESSCA score ($r = 0.4950$, $p = 0.06$). Cognitive performance was screened by the MoCA, where the mean score was 18.9 ± 4.3 points ($n = 11$) and anxiety/depression by PHQ-9, with a mean score of 10.1 ± 5.8 points ($n = 11$). Main demographic and genetics findings are summarized in Table 1 and clinical findings were plotted by frequency (Fig. 2). For additional individual-based clinical findings, see the supplementary data (Table S1).

ATXN3 Allelic Distribution

A total of 682 alleles from 341 ataxic patients were identified by PCR and TP-PCR (Fig. 3). After excluding family members from both SCA3 families (11 individuals) and non-SCA3 families (77 individuals), we identified seven pathogenic alleles ranging from 64 to 75 CAG repeats (1.38%). The remaining 499 normal *ATXN3* alleles ranged from 12 to 38 CAG repeats, with the 23-repeat allele the most common

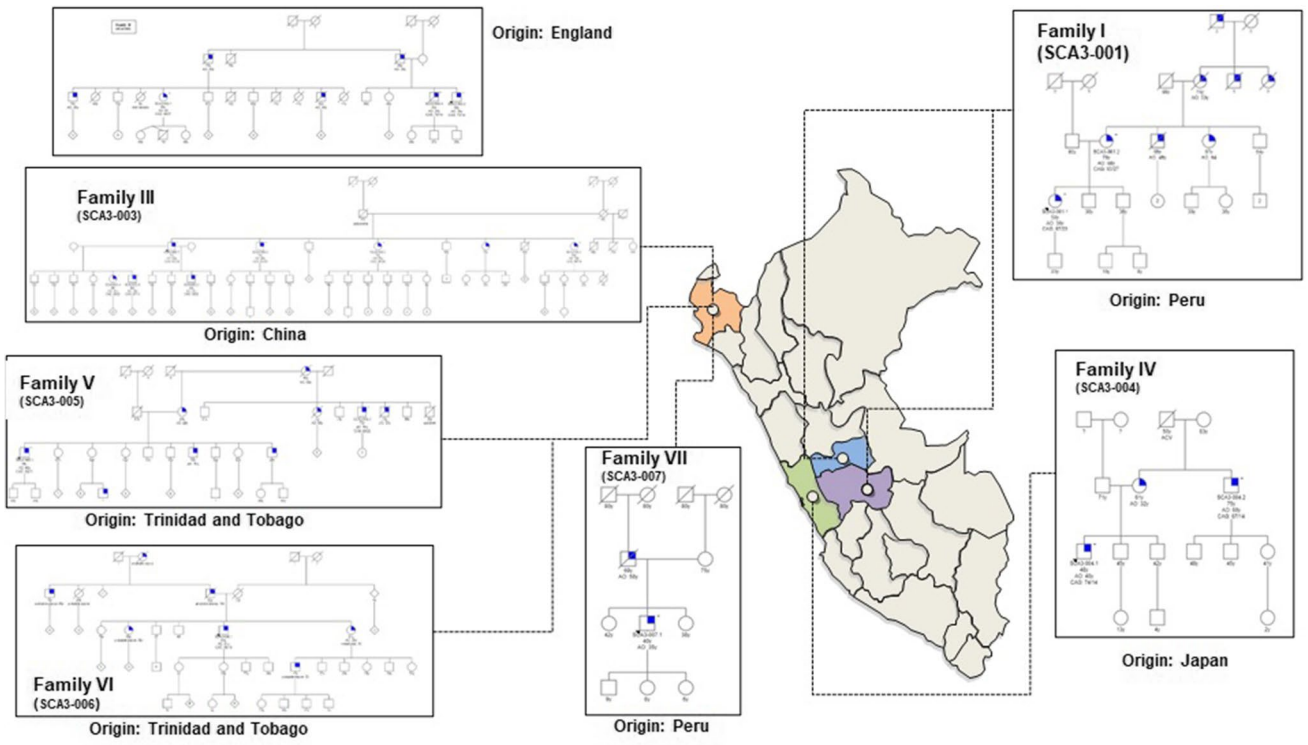


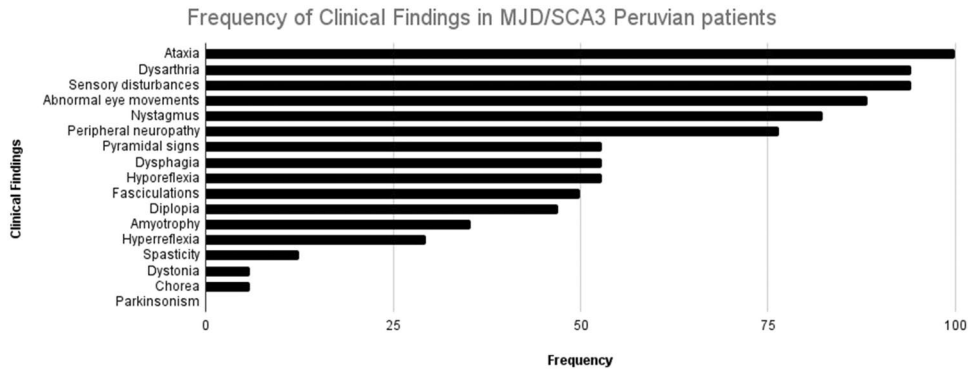
Fig. 1 MJD/SCA3 families in Peru by geographical distribution and origin of ancillary affected family member. *Individuals with available *ATXN3* genotype

Table 1 Main demographic and genetics findings

Family	Individual	Ethnicity	Age at recruitment (years), sex	Age at onset(years)	Age at last follow-up	Time for diagnosis (years)	*ATXN3*-(CAG)n	Family history	Type of transmission	Initial symptom	SARA	NESSCA	MoCA	PHQ-9
SCA3-001	**1.1**	**Mestizo**	**46, female**	**33**	**50**	**14**	**67/23**	**Yes**	**Maternal**	**Numbness in legs**	**19.5**	**20**	**24**	**9**
	1.2	Mestizo	70, female	32	71	38	65/27	Yes	Maternal	Gait ataxia	19	14	13	18
SCA3-002	2.1	Mestizo	68, female	55	68	13	68/27	Yes	Paternal	Gait ataxia	21	15	15	11
	2.2	**Mestizo**	**45, male**	**20**	**45**	**30**	**73/14**	**Yes**	**Paternal**	**Gait ataxia**	**NA**	**NA**	**NA**	**NA**
	2.3	Mestizo	53, male	30	53	23	74/14	Yes	Paternal	Gait ataxia	NA	NA	NA	NA
SCA-003	3.1	Mestizo	63, female	58	66	5	67/27	Yes	Maternal	Gait ataxia	6.5	5	25	6
	3.2	Mestizo	74, female	60	74	14	61/28	Yes	Maternal	Gait ataxia	10	17	13	0
	3.3	Mestizo	76, male	48	76	28	68/26	Yes	Paternal	Gait ataxia	12	10	22	5
	3.4	Mestizo	48, female	47	48	1	68/23	Yes	Paternal	Gait ataxia	3	5	23	7
	3.5	Mestizo	51, male	48	51	3	69/23	Yes	Paternal	Gait ataxia	7	9	17	8
	3.6	Mestizo	39, male	38	46	1	67/23	Yes	Paternal	Gait ataxia	3	4	NA	NA
	3.7	**Mestizo**	**73, male**	**63**	**77**	**13**	**64/14**	**Yes**	**Maternal**	**Gait ataxia**	**14**	**10**	**NA**	**NA**
SCA3-004	**4.1**	**Mestizo**	**45, male**	**37**	**49**	**12**	**74/14**	**Yes**	**Maternal**	**Tingling in right leg and gait ataxia**	**8**	**12**	**21**	**12**
	4.2	Mestizo	74, male	60	76	14	67/14	Yes	Paternal	Gait ataxia	25	16	NA	19
SCA3-005	**5.1**	**Mestizo**	**51, male**	**45**	**71**	**12**	**68/22**	**Yes**	**Maternal**	**Gait ataxia**	**223**	**18**	**18**	**16**
	5.2	Mestizo	65, male	64	65	1	69/23	Yes	Maternal	Gait ataxia	9	NA	13	NA
SCA3-006	**6.1**	**Mestizo**	**63, male**	**61**	**65**	**3**	**69/15**	**Yes**	**Paternal**	**Gait ataxia**	**9.5**	**12**	**NA**	**NA**
SCA3-007	**7.1**	**Mestizo**	**40, male**	**35**	**40**	**6**	**75/23**	**Yes**	**Paternal**	**Diplopia**	**6**	**10**	**NA**	**NA**

Mestizo: admixed ethnicity; for Peruvians, it means predominance of Amerindian component combined with European and Asian ancestries

NA: not available. Probands of each MJD/SCA3 family are highlighted in bold



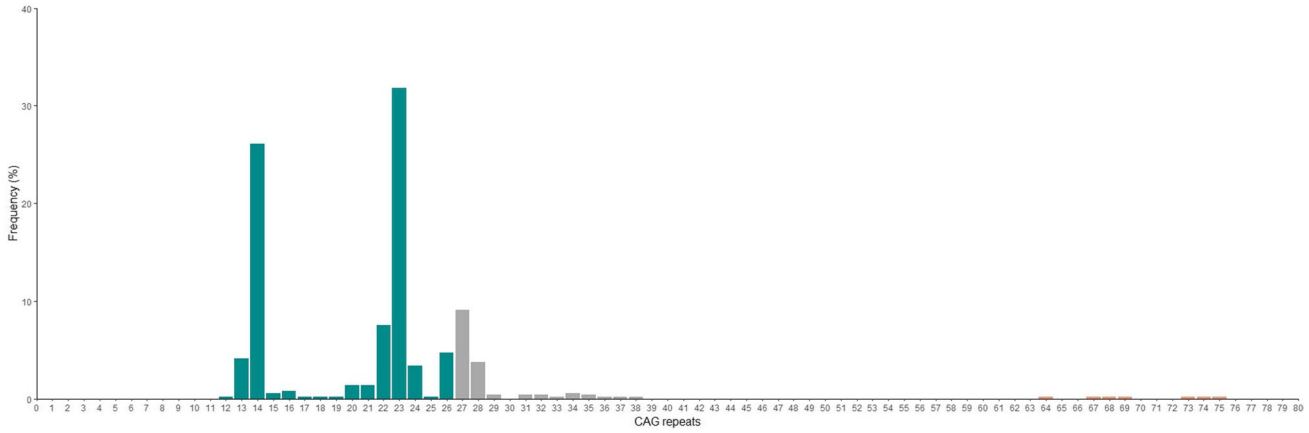


Fig. 2 Frequency of clinical findings in MJD/SCA3 Peruvian patients

Fig. 3 ATXN3 allelic distribution. Cyan bars, normal alleles; gray bars, large normal alleles; orange bars, pathogenic alleles. Mean: 22.1 CAG. Median: 23 CAG. Mode: 23 CAG

(31.8%) followed by 14-repeat allele (26.1%). Moreover, we found 80 LN alleles counting for about 15.8% of the total of alleles.

Discussion

Our findings suggest that MJD/SCA3 is rare in the Peruvian population, with a predominantly foreign origin. We found 18 MJD/SCA3 cases from seven different families, with a relative frequency of 5% of all ataxic cases with clinical diagnosis of inherited ataxia registered from January 1996 to February 2022 in our center. A recent study reported a relative variable frequency of poly-Q SCAs in Peru, with SCA10 being the most common poly-Q SCA and the most common hereditary ataxia in the country, followed by SCA2, whereas SCA7, SCA6, and SCA1 are rare as well as MJD/SCA3 [22]. Other poly-Q SCAs cases such as SCA12, SCA17, and DRPLA have not been reported in Peru to date. Based on the extended pedigrees, we identified that almost all MJD/SCA3 cases have a foreign origin (Fig. 3; Figures S2–S6). Two families reported that the maternal and paternal ancestors of all affected older individuals were Peruvian and lived in different regions separated by the Andes mountains; thus, they might not share a common ancestor (at least until second-degree); we could not reject either a common ancestor or an external origin in these families (Fig. 3; Figures S1 & S7). This frequency contrasts with the reports from Brazil (59.6%), but is consistent with other Latin American countries, such as Mexico (12%) [14] and Cuba (1.2%) [15]. It should be noted that the remaining countries—Argentina, Chile, Venezuela—have a low sample size ($n < 120$), which could increase the actual MJD/SCA3 frequency. Brazil is the country with the highest MJD/SCA3 due to its ancestry from Azorean island families and their founder effect for MJD/SCA3 in South Brazil [23]. Nevertheless, SCA10 predominates in Latin American countries due to Amerindian ancestry, whereas MJD/SCA3 has a low frequency and restricts additional comparison between populations.

MJD/SCA3 in Peru may have been linked to waves of immigration from Europe and Asia. This phenomenon is considered a powerful force shaping the social organization, evolution, and genetics of populations [24]. Therefore, immigrants can pass on their genes to offspring along with

their traits and even diseases such as MJD/SCA3. At least five out of seven families traced an affected family member coming from England, China, Japan, and Trinidad and Tobago (Fig. 3). During the late 1800s and early 1900s, a considerable number of British emigrants settled in Peru due to the foundation of the European Immigration Society [25]. British immigrants settled mainly at the coast, establishing villages across the country. On the other hand, thousands of male Asian coolies, mainly Chinese, came to Peru during the XIX century as a cheap labor force, to work in agriculture, on guano islands and in railway construction [26]. Due to exploitation and semi-slavery, Chinese workers began rebellions in different estates in the 1870s; hence, landowners abandoned the use of Chinese workers, decreasing the labor force. At the same time, Japanese immigration started as an agreement between the government of Japan and Peru, since Japan was experiencing a demographic crisis, while Peru needed labor for work on agriculture [27]. There is no significant historical information regarding immigration from Trinidad and Tobago to Peru. It should be considered a possibility that affected ancestors might be related among some identified families, especially if they live in the same region.

The clinical features of MJD/SCA3 Peruvian patients were concordant with previous reports [3, 4]. The mean age at onset (46.3 ± 13.3 years) was slightly older than usual (34–40 years) [20]. The diagnostic delay of 15.6 years represents up to three times the diagnostic delay of 5 years recently reported in a Brazilian cohort presumably associated with limited access to genetic testing in Peru being almost exclusively available in the capital city. Neurological examination confirmed cerebellar ataxia as the main and predominant symptom in all patients, accompanied by other frequent symptoms such as dysarthria (94.1%), abnormal eye movements (88.2%), and peripheral neuropathy (70–76.5%). Abnormal eye movements include mainly nystagmus, upward gaze palsy (UGP), slow and dysmetric saccades, and saccadic intrusions and "bulging eyes." UGP, found in 64% of cases, has been proposed as an orienting sign that might distinguish MJD/SCA3 from other SCAs [28, 29]. By contrast, spasticity (12.5%), dystonia (5.9%), and chorea (5.9%) were uncommon symptoms in our cohort compared to others [20]; however, their frequency might be affected by the small sample size collected. Dystonia was detected in only one patient and no cases with parkinsonism were found. Non-motor symptoms such as sensory disturbances (94.1%) and depression (75%) were consistent with other MJD/SCA3 reports [30, 31]. Despite cognitive impairment being suggested by the MoCA score of some MJD/SCA3 patients, none of them underwent a neuropsychology assessment. The phenotypic variation within families was not clearly observed, due to limited affected family members per family; however, the family SCA3-003 reflects a variable phenotypic expression ranging from mild ataxia to severe and disabling ataxic syndrome with spasticity. Since the analyzed cohort included patients experiencing ataxia as the main symptom, we did not include premanifest MJD/SAC3 individuals. A pre-symptomatic diagnosis program for some neurogenetics disorders has been recently locally implemented, with no *ATXN3* carriers identified to date.

Although PCR followed by capillary electrophoresis is the gold standard to genotype *ATXN3* gene due its accuracy to measure alleles, we have been able to determine the length of normal and pathogenic alleles based on reference samples that have been genotyped previously by this method. Only 18 pathogenic alleles from 61 to 75 CAG repeats were identified (2.6%) of the total of 682 genotyped alleles. The allelic distribution also showed the 23-repeat CAG allele as the most frequent (32.1%) followed by the 14-repeat CAG allele (26%). Normal alleles ranged from 12 to 38 repeats, which is similar to Caucasian [32, 33], Asian [21, 34], and Latin American [14] populations. We also found that 14.8% of the total of alleles corresponded to the LN subgroup, which were carried by MJD/SCA3 patients and non-MJD/SCA3 patients. This result is consistent with a previous report in Peru [35], and reinforces the lack of correlation between the frequency of LN alleles and the frequency of clinical MJD/SCA3 originally suggested previously in the literature [21].

Conclusion

In conclusion, MJD/SCA3 is very rare in Peru. Almost all affected families have a mutation that originated abroad, which suggests that this was due to an ancestor's migration. Patients with MJD/SCA3 manifested the common phenotype reported in the literature. *ATXN3* allelic distribution in patients with non-MJD/SCA3 ataxia is similar to the distribution in normal individuals around the world, whereas LN allele frequency reinforces no correlation with the frequency of MJD/SCA3. Longitudinal studies should be performed in order to assess the evolution of the phenotype across the time and haplotype studies are required to confirm the foreign origin of MJD/SCA3 in the Peruvian cohort.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s12311-022-01491-4.

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Author Contribution All authors read and approved the final manuscript. All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Lesly Solis-Ponce, Elison Sarapura-Castro, Karina Milla-Neyra, Maryenela Illanes-Manrique, Pilar Mazzetti, Ismael Araujo-Aliaga, Olimpio Ortega, Carla Manrique-Enciso, Diana Cubas-Montecino, Maria Luiza Saraiva-Pereira, Laura B. Jardim, and Mario Cornejo-Olivas. The first draft of the manuscript was written by Ismael Araujo-Aliaga, Lesly Solis-Ponce, and Mario Cornejo-Olivas, and all authors commented on later versions of the manuscript. All authors read and approved the final version of the manuscript.

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Data Availability The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

Declarations

Ethical Approval The study was approved by the Ethics Committee at INCN, called the "*Comité Institucional de Ética en Investigación del Instituto Nacional de Ciencias Neurológicas*," IRB number 486–2018-CIEI-INCN. All patients provided written informed consent for use of their genetic and clinical data for anonymized research studies at the time of their genetic testing. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this work is consistent with those guidelines.

Competing Interests The authors declare no competing interests.

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