Increased frequency of *ATXN1*, *ATXN2* and *HTT* intermediate repeats in frontotemporal dementia and Alzheimer´s disease.

Irene Rosas, MSc[1], Carmen Martínez, MD[2,3], Jordi Clarimón, PhD[4,5], Alberto Lleó,MD,PhD[4,5], Ignacio Illán-Gala, MD,PhD,[4,5], Oriol Dols-Icardo, PhD[4,5],Barbara Borroni MD, PhD[6], Maria Rosário Almeida PhD[7], Julie van der Zee PhD[8,9], Christine Van Broeckhoven PhD, DSc[8,9],on behalf of the EU EOD Consortium, Amalia C. Bruni, MD [10], Maria Anfossi[10], PhD, Livia Bernardi[10], PhD, Raffaele Maletta, MD[10], María Serpente,PhD[11,12], Daniela Galimberti, PhD[11,12], Elio Scarpini, MD[11,12], Giacomina Rossi, PhD[13], Paola Caroppo PhD[13], Luisa Benussi, PhD[14], Roberta Ghidoni ,PhD[14], Giuliano Binetti, MD[14], Benedetta Nacmias,PhD[15], Sandro Sorbi,MD[15], Irene Piaceri,PhD[15], Silvia Bagnoli,PhD[15], Anna Antonell, PhD[16], Raquel Sánchez-Valle MD, PhD[16], Beatriz De la Casa-Fages,MD,PhD[17], Francisco Grandas,MD,PhD[17], Mónica Diez-Fairen MSc,[18,19], Pau Pastor, MD, PhD[18,19], Raffaele Ferrari, PhD[20]on behalf of IFGC, Victoria Álvarez, PhD [1,3], Manuel Menéndez-González, MD, PhD [21,3]

1- Laboratorio de Genética. Hospital Universitario Central de Asturia,.33011,Oviedo, Spain.
2- Servicio de Neurología. Hospital Universitario de Cabueñes,3339,. Gijón, Spain.
3- Instituto de Investigación Sanitaria del Principado de Asturias -ISPA, 33011, Oviedo, Spain.
4-Department of Neurology, IIB Sant Pau, Hospital de la Santa Creu i Sant Pau, Universitat Autónoma de Barcelona, 08025, Barcelona.
5-Center for Networker Biomedical Research in Neurodegenerative Diseases (CIBERNED)
6-Centre for Neurodegenerative Disorders, Department of Clinical and Experimental Sciences, University of Brescia, Brescia, Italy.
7-Center for Neuroscience and Cell Biology, University of Coimbra, 3004-504, Coimbra, Portugal.
8- Neurodegenerative Brain Diseases Group, Center for Molecular Neurology, VIB, Antwerp, Belgium
9- Laboratory of Neurogenetics, Institute Born-Bunge, University of Antwerp, Antwerp, Belgium.
10-Regional Neurogenetic Centre, ASP CZ, Lamezia Terme, Catanzaro, Italy.
11-Deept. of Pathophysiology and Transplantation, "Dino Ferrari" Center, University of Milan.
12-Fondazione IRCCS Ca' Granda, Ospedale Policlinico, Neurodegenerative Diseases Unit, Milan, Italy
13-Fondazione IRCCS Istituto Neurologico Carlo Besta , Milan, Italy.
14-Fatebenefratelli Biobank Molecular Markers Laboratory. IRCCS Istituto Centro San Giovanni di Dio-Fatebenefratelli, Brescia, Italy.
15- Department of Neuroscience, Psychology, Drug Research and Child Health University of Florence Azienda Ospedaliero-Universitaria CareggiViale, Florence, Italy.
16- Alzheimer's disease and other cognitive disorders Unit. Hospital Clínic. Fundació Clínic per a la Recerca Biomèdica. Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS). Barcelona, Spain.
17-Movement Disorders Unit, Neurology Department, Hospital General Universitario Gregorio Maranon, Madrid, Spain.
18- Movement Disorders Unit, Department of Neurology (P.P.), University Hospital Mutua de Terrassa, Terrasa, Barcelona, Spain.
19-Fundació per la Recerca Biomèdica i Social Mútua de Terrassa, Terrassa, Barcelona, Spain.
20-Department of Neurodegenerative Disease, University College London, Institute of Neurology, Queen Square, London,WC1B 5EH, UK.
21- Servicio de Neurología. Hospital Universitario Central de Asturias.33011, Oviedo , Spain.

Corresponding author :
Dra. Victoria Álvarez ,PhD
Laboratorio de Genética . Hospital Universitario Central de Asturias, Avenida de Roma s/n, 33011 , Oviedo.
Email: victoria.alvarez@sespa.es

Word count for the manuscript: 3042

Keywords

Neurodegeneration, CAG repeats, intermediate alleles.

Abstract

Background

The aim of this study was to determine the frequency of intermediate alleles (IAs) in *ATXN1, ATXN2* and *HTT* in patients with neurodegenerative diseases.

Methods

This is a multicentric study that included 1126 Alzheimer disease (AD) , 433 frontotemporal dementia (FTD) and 610 Parkinson´s disease (PD) patients. In all cohorts, we genotyped CAG repeats in *ATXN1* and *ATXN2* genes. Additionally, in FTD cohort we genotyped CAG repeats in *HTT* gene.

Results

In the overall FTD cohort, the frequency of *HTT* IAs was significantly higher in cases comparatively to controls (6.9% vs. 2.9%; p=0.007.; OR:2.45 (1.30-4.62). The frequency was even higher for *c9orf72* expansion negative individuals (7.4% vs. 2.9%; p=0.008, OR:2.63 (1.31-5.26). BvFTD and PNFA groups showed a significant difference in the frequency of *HTT IAs (bvFTD 6.6% vs 2.9%, p=0.032, OR:2.31(1.12-4.76);PNFA 13.2% vs 2.9% p=0.0025, OR:5.01(1.94-12.92).* For *the ATXN2* gene, we observed an IAs increased frequency in AD cases compared to controls (AD 4.1% vs 1.8% p=0.024 OR:2.37(1.15-4.87). For the *ATXN1* gene, we found a significant increase of IAs in PNFA patients vs control group (15.1% *vs* 6.7% p=0.034; OR: 2.48(1.08-5.69).

Conclusions

Our work suggests that intermediate repeats in *HTT*, *ATXN1* and *ATXN2* may contribute to increase risk for AD as well as bvFTD and PNFA pathogenesis.

1. Introduction

Frontotemporal dementia (FTD) is a group of cognitive disorders due to degeneration in the frontal and temporal lobes that present heterogeneous clinical, pathological and genetic features. Clinically, FTD is subdivided in behavioral and language variants, more specifically behavioural FTD (bvFTD) or progressive non-fluent aphasia (PNFA) or fluent progressive aphasia (semantic dementia, SD). Familial FTD, i.e. patients with a familial history and an autosomal dominant form of inheritance, is seen in 10-27% of the cases.[1] Several FTD-causative mutations have been identified in different genes including *C9orf72, GRN, MAPT* and *TBK1* [1]. Some of these mutations have also been identified in amyotrophic lateral sclerosis (ALS), demonstrating a genetic overlap between both disorders. In addition, other genetic factors may act as modifiers of the disease in FTD and MND [2]. A pathogenic expansion in *C9orf72* is the most common genetic cause of FTD and ALS, accounting for 29% of familial FTD [1]. The clinical phenotype of the *C9orf72* expansion is very heterogeneous, even within the same family, being the bvFTD subtype the most common clinical presentation, but FTD-MND, MND (motor neuron disease) and PNFA presentations are also common [3]. Moreover, *C9orf72* repeat expansions have also been described in cases clinically diagnosed with Alzheimer disease (AD), Parkinson Disease (PD) and Huntington Disease (HD) among others [4].

Spinocerebellar ataxia type 1 (SCA1), Spinocerebellar ataxia type 2 (SCA2) and HD are autosomal dominant progressive neurodegenerative diseases, caused by the presence of an abnormally expanded CAG repeats (polyglutamine, polyQ) in *ATXN1, ATXN2* and *Huntingtin (HTT)* genes, respectively. Expansions in these genes may fall in an intermediate range (intermediate alleles, IAs) between normal and pathological range. IAs have the property to increase the length in the offspring, reaching the pathological range in some cases. IAs in the *HTT* gene are defined as CAG repeats between 27 and 35; IAs in the *ATXN1* gene range between 27 and 33 repeats; and IAs in the *ATXN2* gene range between 33 and 38 repeats. In the last years, several studies have assessed the potential effect of IAs in several neurodegenerative diseases. For instance, previous results from our group suggest that *HTT* IAs might have a role in the pathogenesis of AD [5]. In addition, IAs at *ATXN1* gene could act as a disease risk factor for ALS, mostly among *C9orf72* expansion carriers [6]. The presence of IAs in the *ATXN2* gene has been associated with an increased risk of ALS [6-8] but no significant association was found between ALS and IAs in *HTT*.[7,9] Also, no differences were found between the *ATXN2 IAs* frequency of in FTD and healthy controls [10,11] but it has been suggested that IAs in *ATXN2 c*ould act as a FTD phenotype modifier in *C9orf72* expansion carriers.[12] At last, it has been demonstrated that intermediate *C9orf72* expansion has a risk effect in familial/sporadic FTD.[13,14] All this taken together suggests IAs might play a major role in neurodegeneration.

The aim of this study was to determine the frequency of IAS in *ATXN-1, ATXN-2* and *HTT* in patients with neurodegenerative diseases such as AD, PD and FTD cases with or without *c9orf72* expansions.

2. Materials and methods.

2.1 Patients and controls.

2.1-Study Design

This is a multicentre genetic study based on samples obtained from biobanks. Clinical data was retrospectively collected from medical records. We used three cohorts of samples clinically diagnosed with AD, FTD and PD. In all cohorts, we genotyped CAG repeats in the *ATXN1* and *ATXN2* genes. We

also genotyped CAG repeats in the *HTT* gene for the FTD cohort. In the AD and PD cohorts *HTT* CAG repeats had been genotyped in a previous work.

2.2-Patients and medical records

All patients were Caucasian. Our FTD cohort included 433 unrelated patients (286 from Spain, 101 from Italy, 26 from Belgium and 20 from Portugal) diagnosed with behavioural variant FTD (n=244), semantic dementia (n=31), progressive nonfluent aphasia (n=53), or FTD-MND (n=44), according to the behavioural and language variants diagnostic criteria [15] and [16]. In 61 patients, FTD clinical presentation was unspecified and 176 patients were carriers of the *C9orf72* expansion. Family history of dementia were reported in 88.4% of C*9orf72* expansion carriers and 41.3% in non-carriers. For FTD patients, we collected data of age at onset, phenotype and family history.

Additionally, we analyzed an AD cohort composed of 1126 patients clinically diagnosed according to NIH-AA criteria [17] and a cohort of 610 with Parkinson's disease according to the MDS criteria [18]. These cohorts had been used in a previous work [5] where we analyzed the prevalence of *IAs* in the *HTT* gene in neurodegenerative diseases.

All the patients were ascertained at Hospital Universitario Central de Asturias (Spain); Hospital Santa Creu i Sant Pau (Spain); Centre for Neurodegenerative Disorders- University of Brescia (Brescia, Italy); Center for Neuroscience and Cell Biology, University of Coimbra (Coimbra, Portugal); Center for Molecular Neurology, VIB- University of Antwerp (Antwerp, Belgium), Regional Neurogenetic Centre, ASP CZ, Lamezia Terme (Catanzaro, Italy); Fondazione IRCCS Ca' Granda, Ospedale Policlinico (Milan, Italy).; RCCS Istituto Centro San Giovanni di Dio- Fatebenefratelli (Brescia, Italy), University of Florence Azienda Ospedaliero (Florence,Italy), Hospital Clínic (Barcelona, Spain); Hospital Gregorio Marañón (Madrid, Spain); IRCCS Istituto Fondazione IRCCS Istituto Neurologico Carlo Besta, (Brescia, Italy) and University Hospital Mutua de Terrassa, (Terrassa, Barcelona, Spain). The control group was a cohort of unrelated Caucasian individuals (n=509) free of neurodegenerative disease. They were recruited through the Health Community Service (elderly subjects who agreed to participate).

2.2 Genetic analysis

Genomic DNA was isolated from peripheral blood according to standard procedures. In the FTD cohort, *HTT, ATXN1 and ATXN2* CAG repeat length were determined by polymerase chain reaction (PCR) with fluorescent-labeled primers with capillary electrophoresis using a ABI 3130X automated DNA sequencer and the Gene Mapper version 4.0 software (Applied Biosystems). As a reference, we sequenced several samples with different *HTT, ATXN-1* and *ATXN-2* CAG alleles.

C9orf72 expansion methodology

C9orf72 genetic status was determined by triple repeat primed polymerase chain reaction (TP-PCR) using standard protocols.[19-21] FTD patients were assessed for the presence of the *C9orf72* expansion. All the *C9orf72* positive subjects in our cohort had more than 30 repeats. All the patients and controls were also genotyped for the Apolipoprotein E (*APOE*) alleles (E2, E3, and E4) by real time PCR using commercially available Taqman assays (Applied Biosystems) or by PCR and enzyme digestion as previously described [22].

2.3 Statistical analyses

The Chi^2 and Fisher's exact tests, with the Bonferroni adjustment, were used to compare the frequency of *ATXN-1, ATXN-2 and HTT* intermediate alleles between patient's group and controls. In order to analyze the CAG-repeats distribution among the different groups, the Kruskal-Wallis test was used to correct for samples that did not display a normal distribution, followed by Dunn´s post hoc test whenever that was appropriate. The parametric Student t-test was performed to analyze the correlation between IA and age of onset.

The statistical analyses were performed using R statistical Software (version 3,4,4) and SPSS (version 17).

2.4 Standard protocols approvals , registrations and patient consents

All the patients and controls gave informed consent to participate in the study which was approved by the Ethical Committees of the participating centers.

3. Results

Table 1 displays the demographic data of patients and controls and clinical presentation of FTD patients. In all groups, *HTT* alleles with 17 and 18 CAG-repeats were the most frequent . In the IAs

distribution, the most frequent repeats number was 27 CAG-repeats, whilst the longest was 34 **(*Figure 1)***. For the *ATXN2* gene and *ATXN1* genes, the most prevalent alleles are 22 CAG and 29 - 30 CAG repeats, respectively (***Figure 2 and Figure 3).***

Using Kruskal-Wallis test, no differences were found in the distribution of normal *HTT, ATXN1 and ATXN2* alleles between groups of patients and controls. For intermediate alleles, no difference in the *HTT, ATXN1 and ATXN2* allele distribution was detected likely due to the small number of patients carrying IAs (data not shown).

The frequency of IAs in *HTT, ATXN1 and ATXN*2 gene in control cohort was similar to it found in other european populations.

Table 1- Subjects demographic and clinical data

Group	N	Male (%)	Age at examination (controls) / Age at onset (patients)rrhh
Controls	509	234(46%)	71.14±6.42
FTD	433	238 (55%)	61.58±10.34
FTD *C9orf72* carriers	176	98(55.7%)	57.71±10.01
FTD *C9orf72* non carriers	257	140(54,5%)	63.92±9.83
bvFTD **pnFA** **SD** **FTD-MND** **Unspecified**	244 53 31 44 61	145(59,4%) 23(43,4%) 16(51,6%) 29(65,9%) 25(41%)	60.38±10.31 66.17±7.79 62.73±9.38 59.58±11.50 62.65±10.99
AD	1126	337(30%)	74.32±9.67
PD	610	315(52%)	59.85±13.6

Table 2 summarizes the frequency of IAs *HTT* alleles. In the FTD cohort, 6.9% of patients harbored *HTT* intermediate alleles, which was significantly higher than for controls (2.9%; p=0.007; OR:2.45 (1.30-4.62)). Statistically significant differences were also observed in the *C9orf72* expansion non-carriers subgroup (7.4% vs. 2.9% in controls, p=0.008, OR:2.63 (1.31-5.26)). No statistically significant differences were observed between *C9orf72* carriers and controls nor between expansion carriers and non-carriers.Among the clinical subgroups, bvFTD and PNFA groups showed a significant difference in the frequency of *HTT IAs* (bvFTD 6.6% vs 2.9%, p=0.032, OR:2.31(1.12-4.76); PNFA 13.2% vs 2.9% p=0.0025, OR:5.01(1.94-12.92). All PNFA patients with *HTT* IAs were negative for the *C9orf72* expansion, while in the bvFTD group out of 16 IAs carriers, 8 (50%) were *c9orf72* non-carriers.

Table 2. Frequencies of *HTT i*ntermediate alleles.

Group	Carriers of *HTT* IAs	p-value	Bonferroni

Controls	15 (2.9%)		
FTD	30(6.9%)	p=0.007*	
FTD *c9orf72* carriers	11 (6.3%)	p=0.08	[a]p=0.228
FTD *c9orf72* non carriers	19 (7..4%)	p=0.008*	[a]p=0.019
BvFTD	16 (6.6%)	p=0.032*	p=0.438
pnFA	7 (13.2%)	p=0.0025*	p=0.013
SD	1 (3.2%)	p=1	p=1
FTD-MND	3 (6.8%)	p=0.166	p=1
FTD unspecified	3 (4.9%)	p=0.427	p=1

**- patient cohort vs controls*
a- p adjusted comparing carriers, non carriers and controls.

For the *ATXN2* gene, we observed a significant increased frequency of intermediate alleles (≥27 repeats) in AD compared to the control group (AD 4.1% vs 1.8% p=0.024 OR:2.37(1.15-4.87). In the FTD and PD cohorts, the IAs frequency was not statistically different to that observed in the control group **(*Table 3*).** We examined the presence of ≥ 29 CAG repeats alleles in AD and FTD groups and, although the association was not statistically significant, there was an increased frequency of IAs ≥29 repeats in both patient groups (AD 0.8% vs 0.4% p=0.518; FTD 0.9% vs 0.4% p=0.42).(data not shown)

Table 3. Frequencies of *ATXN2* intermediate alleles.

Group	Carriers of *ATXN2* IAs	p-value	OR(95%CI)	Bonferroni
Controls	≥27: 9 (1.8%)			
FTD	≥27: 16(3.7%)	p=0.103		[a]p= 0.544
FTD *c9orf72* carriers	≥27: 6(3.4%)	p=0.231		[b]p=1
FTD *c9orf72* non carriers	≥27: 10 (3.9%)	p=0.124		[b]p=1

AD	≥27: 46 (4.1%)	p=0.024*		[a]p=0.077 [b]p=0.128
PD	≥27: 13 (2.1%)	p=0.823		[a]p=1

** patients vs controls*
a- p adjusted comparing controls, FTD, AD and PD groups
b-p adjusted comparing controls , c9orf72 carriers, c9orf72 non carriers, AD and PD groups.

For the *ATXN1* CAG repeats, we considered 33 as a cut-off between normal and intermediate alleles length. The frequency of *ATXN1* IAs was similar in all patient groups, but we found a significant increase of these alleles in PNFA patients vs control group (15.1% *vs* 6.7% p=0.034; OR: 2.48(1.08-5.69)) ***(Table 4)***.

Table 4. Frequencies of *ATXN-1* intermediate alleles.

Group	Carriers of *ATXN-1* IAs	p-value	Bonferroni
Controls	34 (6.7%)		
FTD	33 (7.6%)	p=0.663	
FTD C*9orf72* carriers	13(7.4%)	p=0.887	
FTD C*9orf72* non carriers	20(7.8%)	p=0.680	
bvFTD	15(6.1%)	p=0..920	p=1
pnFA	8(15.1%)	p=0.034*	[a]p=0.352
SD	2(6.5%)	p=1	p=1

FTD-MND	5(11.4%)	p=0.351	p=1
Unspecified	3(4.9%)	p=0.786	p=1
AD	81 (7.2%)	p=0.791	
PD	58(9.5%)	p=0.108	

** patients vs controls*
a- p adjusted comparing controls, bvFTD, PNFA; SD; FTD-MND and FTD unspecified.

We did not find differences in the mean age of onset between carriers and non-carriers of *HTT, ATXN2* and *ATXN1* IAs in any patient´s group (data no shown) .
As expected, the frequency of the *APOE*-E4 allele was significantly higher in AD patients compared to controls (0.26 vs 0.089 p<0.0001; OR= 3.73, 95% CI=2.83-4.41). The risk of AD was significantly increased in patients with E4/E4 genotype (p<0.0009; OR= 13.011). In the FTD and PD groups, the *APOE* allele and genotype frequencies were similar to the control group. In FTD patients, the frequency of *HTT, ATXN2 and ATXN1* IAs and age at onset did not differ between *APOE*-E4 carriers and non-carriers. Similarly, in the AD and PD group we did not observe differences in the *ATXN2* IAs frequency and age at onset between E4 positive and E4 negative patients (data not shown).

4. Discussion

Recently, we have described that the *HTT I*As might play a role in the pathogenesis of AD because their frequency was significantly higher among AD patients than in controls [5]. In that study, no significant difference was observed between FTD patients and controls. There was, however, a higher number of *HTT* IAs in FTD patients than in controls, just not reaching statistical significance probably due to the relatively small size of the FTD cohort.
In the present study, we aimed to increase the sample size and examined the frequency of intermediate *HTT, ATXN1 , ATXN2 alleles* in a larger, multicentric pool of FTD cases, including 176 *C9ORF72* expansion carriers and 257 non expansion carriers. Additionally, we also analyzed the frequency of *ATXN2 and ATXN1* intermediate alleles in our AD and PD cohorts. We identified a significant association of *HTT* IAs with disease risk for FTD (p=0.007) and a significant increase of *ATXN2 IA*s in AD patients compared to the control cohort (p=0.024). Previous studies had evaluated the role of CAG repeat size in several neurodegenerative and psychiatric diseases. A larger study using data from the European Huntington´s Disease Registry database showed that elderly *HTT* IAs carriers had more chorea and faster cognitive decline than controls [23]. Also, a population based study found that IAs carriers have a higher risk of manifesting apathy and suicidal ideation,[24] while an "U- relationship" (U-shaped relation) has been found between the number of repeats and the risk of suffering depression [25]. Several studies have confirmed a significant association between *ATXN2* IAs and ALS suggesting that the 29-33 range of *ATXN2* repeat alleles is a strong risk factor for developing ALS but also a phenotypic modifier.[9,26] Our results are in agreement with previous reports that suggest no

association of *ATXN2 IAs* with FTD disease risk but, to our knowledge, our current work is the first report of an association of IAs in *HTT* and *ATXN2* genes with FTD and AD risk, respectively. HD and SCA2 are polyglutamine diseases which are characterized by progressive motor symptoms, psychiatric disturbances, and cognitive deterioration. The polyglutamine domains of the associated proteins act as critical regulators of fundamental cellular processes such as transcriptional regulation, mitochondrial energy production and autophagy [7,27,28], pathways which have been associated with aging and age-related disorders such as AD [29,30]. Interestingly, amyloid beta precursor protein splicing patterns in the brain of SCA2 patients resemble those seen in AD brains [31].

A recent study reported that CAG repeat size in the *ATXN1* gene is associated with different clinical features in AD such as memory, attention and atrophy of the medial temporal lobes [32]. In our AD cohort, association of *ATXN1* repeats with AD disease risk, age at onset or APOE genotype was not observed. Concerning the *HTT* IAs, we found statistically significant differences between *C9orf72* non-carriers and controls (p=0.008). Among clinical subgroups, bvFTD and PNFA groups also showed significantly different frequencies of *HTT* IAs compared to controls *(bvFTD 6.6% vs 2.9%, p=0,032 ; PNFA 13.2% vs 2.9% p=0.0025)*. For the *ATXN1* CAG repeats, we also observed a significant increase of IA frequency in the PNFA group. In the PNFA group, all *HTT* IA carriers and 7 out of 8 ATXN1 IA carriers were negative for the *C9ORF72* expansion.

Since AD and most of the *C9orf72*-negative bvFTD and PNFA cases are known to be tauopathies, whereas all *C9orf72* positive FTDs and most *C9orf72* negative SDs are "TDPopathies" (with deposits of protein TDP43), our results suggest that IAs in the *ATXN1, ATXN2* and *HTT* genes may play a more specific role in tauopathies (**Figure 4)**. Interestingly, HD has also been proposed to be a tauopathy[33]: first, the mutant HTT protein alters tau splicing, phosphorylation, oligomerization and subcellular localization [34,35]; second, patients with HD present aggregated tau inclusions within various structures of the brain, including those with young-onset [36,37]; and third, the MAPT H2 haplotype influences the cognitive function of HD patients [37].

Although, the results of our work suggest a potential link between IAs with tauopathies, a more general role in neurodegenerative conditions for some of these genes cannot be excluded. In fact, it has been described that *ATXN1* IAs are a risk factor for ALS, mostly in *C9ORF72* expansion carriers [38]. It will be crucial to know whether IAs for *HTT* , *ATXN2* and ATXN1 can contribute to neurodegeneration, and this can only be solved when neuropathological studies on polyQ deposits in IAs carriers will be performed. However, there are patients with low number of *C9orf72* gene repeats who showed clinical symptoms along with pathological lesions without TDP-43 inclusions [39]. Moreover, the only autopsy confirmed report with diagnosis of HD had 29 CAG repeats [40] with some neuropathological changes compatible with HD but no huntingtin intranuclear inclusions.

The main limitation of our study is that it is a retrospective and multicentric study where the diagnosis of cases is not supported by biomarkers or neuropathology to confirm the diagnoses. Also, there is a small sample size for some clinical subtypes of FTD as PNFA or SD, which do not allow to obtain more robust conclusions for these groups.

The genetic architecture of FTD and AD is complex and many genetic variants might differently module disease pathogenesis. These variants, including the number of CAG repeats, might have a synergistic effect in the disease onset and progression, and on determining of the clinical phenotype. In this respect, and provided replication in larger patients cohorts, findings of our current work might suggest that repeats in *HTT*, *ATXN1* and *ATXN2* may modulate and contribute to AD as well as *C9orf72* expansion negative bvFTD and PNFA pathogenesis. These results also supports the inference that there may be communal pathways for a cluster of neurodegenerative diseases linked by tau dysfunction.

Acknowledgements: We are indebted to all the patients and their families for their participation. Authors wish to thank Fundación Parkinson Asturias-Obra Social Cajastur for their support. We thank the IFGC (https://ifgcsite.wordpress.com/), the EUEOD and DEGESCO for facilitating this study.

Study Funding: Irene Rosas is supported by a grant from Fundación Jose Luis Castaño-SEQC This study is supported by grant PI 15/00878 (Fondos Feder) to VA. O. Dols-Icardo is funded by Departament de Salut de la Generalitat de Catalunya, Pla strategic de recerca i innovació en salut (PERIS) 2016-2020 (SLT002/16/00040). I. Illán-Gala is supported by the Rio Hortega grant (CM17/00074) from "Acción Estratégica en Salud 2013-2016" and the European Social Fund. JC and

VA research´s groups are members of Dementia Genetics Spanish Consortium (DEGESCO) . CVB and JvdZ were in part funded by the Flemish Government initiated Impulse Program on Networks for Dementia Research (VIND) and the Methusalem Excellence Program; the Research Foundation Flanders (FWO) and the University of Antwerp Research Fund. LB,GB,RG are supported by the Italian Ministry of Health (Ricerca Corrente). A. Antonell is funded by Departament de Salut de la Generalitat de Catalunya, Pla Estrategic de recerca i innovació en salut (PERIS) 2016-2020 (SLT002/16/00329). RF is supported by Alzheimer's Society (grant number 284).

Disclosure: The authors report no conflicts of interest related with this work.

References

1. Van Mossevelde S, Engelborghs S, van der Zee J, et al. Genotype-phenotype links in frontotemporal lobar degeneration. Nat Rev Neurol 2018;14:363-378 doi:10.1038/s41582-018-0009-8.

2. van Blitterswijk M, Mullen B, Wojtas A, et al. Genetic modifiers in carriers of repeat expansions in the C9ORF72 gene. Mol Neurodegener 2014;9:38 doi: 10.1186/1750-1326-9-38.

3. Simón-Sánchez J, Dopper EG, Cohn-Hokke PE, et al. The clinical and pathological phenotype of C9ORF72 hexanucleotide repeat expansions. Brain 2012;135:723-35 doi:10.1093/brain/awr353

4. Ahmed RM, Devenney EM, Irish M, et al. Neuronal network disintegration: common pathways linking neurodegenerative diseases. J Neurol Neurosurg Psychiatry 2016;87:1234-1241 doi:10.1136/jnnp-2014-308350.

5. Menéndez-González M, Clarimón J, Rosas I, et al. HTT gene intermediate alleles in neurodegeneration: evidence for association with Alzheimer's disease. Neurobiol Aging EPub 28 Nov 2018 doi.org/10.1016/j.neurobiolaging.2018.11.014

6. Conforti FL, Sparato R, Sproviero W, et al. Ataxin-1 and ataxin 2 intermediate-length PolyQ expansions in amyotrophic lateral sclerosis. Neurology 2012;79:2315-20 doi:10.1212/WNL.0b013e318278b618

7. Lee T, Li YR, Chesi A, et al. Evaluating the prevalence of polyglutamine repeat expansions in amyotrophic lateral sclerosis. Neurology 2011;76:2062-5 doi:10.1212/WNL.0b013e31821f444
8. Sproviero W, Shatunov A, Stahl D, et al. ATXN2 trinucleotide repeat length correlates with risk of ALS. Neurobiol Aging 2017;51:178.e1-178.e9 doi:10.1016/j.neurobiolaging.2016.11.010
9. Ramos EM, Keagle P, Gillis T, et al. Prevalence of Huntington's disease gene CAG repeat alleles in sporadic amyotrophic lateral sclerosis patients. Amyotroph Lateral Scler 2012;13:265-9 doi:10.3109/17482968.2011.653573
10. Ross OA, Rutherford NJ, Baker M, et al. Ataxin-2 repeat-length variation and neurodegeneration. Hum Mol Genet 2011;20:3207-12 doi: 10.1093/hmg/ddr227
11. van Blitterswijk M, Mullen B, Heckman MG, et al. Ataxin-2 as potential disease modifier in C9ORF72 expansion carriers. Neurobiol Aging 2014;35:2421.e13-7 doi:10.1016/j.neurobiolaging.2014.04.016
12. Lattante S, Millecamps S, Stevanin G, et al. Contribution of ATXN2 intermediary polyQ expansions in a spectrum of neurodegenerative disorders. Neurology 2014;83:990-5. doi: 10.1212/WNL.0000000000000778.
13. van der Zee J, Gijselinck I, Dillen L, et al A pan-European study of the C9orf72 repeat associated with FTLD: geographic prevalence, genomic instability, and intermediate repeats. Hum Mutat. 2013;34:363-73 doi:10.1002/humu.22244.
14. Benussi L, Rossi G, Glionna M, et al. C9ORF72 hexanucleotide repeat number in frontotemporal lobar degeneration: a genotype-phenotype correlation study. J Alzheimers Dis.2014;38:799-808 doi: 10.3233/JAD-131028.
15. Rascovsky K, Hodges JR, Knopman D, et al. Sensitivity of revised diagnostic criteria for the behavioural variant of frontotemporal dementia. Brain 2011;134:2456-77 doi:10.1093/brain/awr179
16. Gorno-Tempini ML, Hillis AE, Weintraub S, et al. Classification of primary progressive aphasia and its variants. Neurology 2011;76:1006-14 doi:10.1212/WNL.0b013e31821103e6
17. McKhann GM, Knopman DS, Chertkow H, et al. The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement 2011;7:263-9. doi:10.1016/j.jalz.2011.03.005
18. Postuma RB, Berg D, Stern M, et al. MDS clinical diagnostic criteria for Parkinson's disease. Mov Disord 2015;30:1591-601 doi:10.1002/mds.26424.
19. DeJesus-Hernandez M, Mackenzie IR, Boeve BF, et al. Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. Neuron 2011;72:245-56 doi:10.1016/j.neuron.2011.09.011
20. Renton AE, Majounie E, Waite A, et al. A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. Neuron 2011;72:257-68 doi:10.1016/j.neuron.2011.09.010
21. Gijselinck I, Van Langenhove T, van der Zee J, et al. A C9orf72 promoter repeat expansion in a Flanders-Belgian cohort with disorders of the frontotemporal lobar degeneration-amyotrophic lateral sclerosis spectrum: a gene identification study. Lancet Neurol 2012;12:54-65 doi:10.1016/S1474-4422(11)70261-7
22. Hixson JE, Vernier DT. Restriction isotyping of human apolipoprotein E by gene amplification and cleavage with HhaI. J Lipid Res 1990;31:545-8.
23. Cubo E, Ramos-Arroyo MA, Martinez-Horta S, et al. Clinical manifestations of intermediate allele carriers in Huntington disease. Neurology 2016;87:571-8 doi:10.1212/WNL.0000000000002944

24. Killoran A, Biglan KM, Jankovic J, et al. Characterization of the Huntington intermediate CAG repeat expansion phenotype in PHAROS. Neurology 2013;80:2022-7 doi:10.1212/WNL.0b013e318294b304
25. Gardiner SL, van Belzen MJ, Boogaard MW, et al. Huntingtin gene repeat size variations affect risk of lifetime depression. Transl Psychiatry 2017;7:1277 doi:10.1038/s41398-017-0042-1.
26. Chiò A, Calvo A, Moglia C, et al. ATXN2 polyQ intermediate repeats are a modifier of ALS survival. Neurology. 2015 20;84:251-8. doi: 10.1212/WNL.0000000000001159.

27. Ashkenazi A, Bento CF, Ricketts T, et al. Polyglutamine tracts regulate beclin 1-dependent autophagy. Nature 2017;545:108-111 doi: 10.1038/nature22078
28. Hannan AJ. Synaptopathy, circuitopathy and the computational biology of Huntington's disease. BMC Biol 2018;16:71 doi:10.1186/s12915-018-0539-y.
29. Caldeira GL, Ferreira IL, Rego AC. Impaired transcription in Alzheimer's disease: key role in mitochondrial dysfunction and oxidative stress. J Alzheimers Dis 2013;34:115-31 doi:10.3233/JAD-121444.
30. Gardner A, Boles RG. Beyond the serotonin hypothesis: mitochondria, inflammation and neurodegeneration in major depression and affective spectrum disorders. Prog Neuropsychopharmacol Biol Psychiatry 2011;35:730-43 doi:10.1016/j.pnpbp.2010.07.030
31. Li PP, Sun X, Xia G, et al. ATXN2-AS, a gene antisense to ATXN2, is associated with spinocerebellar ataxia type 2 and amyotrophic lateral sclerosis. Ann Neurol 2016;80:600-15 doi: 10.1002/ana.24761
32. Gardiner SL, Harder AVE, Campman YJM, et al. Repeat length variations in ATXN1 and AR modify disease expression in Alzheimer's disease. Neurobiol Aging 2019;73:230.e9-230.e17 doi:10.1016/j.neurobiolaging.2018.09.007
33. Gratuze M, Cisbani G, Cicchetti F, Planel E. Is Huntington's disease a tauopathy? Brain 2016;139:1014-25 doi:10.1093/brain/aww021
34. Blum D, Herrera F, Francelle L, et al. Mutant huntingtin alters Tau phosphorylation and subcellular distribution. Hum Mol Genet 2015;24:76-85 doi:10.1093/hmg/ddu421
35. Fernández-Nogales M, Santos-Galindo M, Hernández IH, et al. Faulty splicing and cytoskeleton abnormalities in Huntington's disease. Brain Pathol 2016;26:772-778 doi: 10.1111/bpa.12430
36. Fernández-Nogales M, Cabrera JR, Santos-Galindo M, et al. Huntington's disease is a four-repeat tauopathy with tau nuclear rods. Nat Med 2014;20:881-5 doi:10.1038/nm.
37. Vuono R, Winder-Rhodes S, de Silva R, et al. The role of tau in the pathological process and clinical expression of Huntington's disease. Brain 2015;138:1907-18 doi:10.1093/brain/awv107
38. Lattante S, Pomponi MG, Conte A, et al. ATXN1 intermediate-length polyglutamine expansions are associated with amyotrophic lateral sclerosis. Neurobiol Aging 2018;64:157.e1-157.e5.doi:10.1016/j.neurobiolaging.2017.11.011.
39. Ng ASL, Tan EK. Intermediate C9orf72 alleles in neurological disorders: does size really matter? J Med Genet 2017;54:591-597 doi: 10.1136/jmedgenet-2017-104752
40. Kenney C, Powell S, Jankovic J. Autopsy-proven Huntington's disease with 29 trinucleotide repeats. Mov Disord 2007;22:127-30.

Figure´s footnotes and title

Figure 1

Title- HTT CAG repeats distribution

Footnote- (A) Distribution of *HTT* CAG repeats in patients and controls. (B) Distribution of *HTT* intermediate alleles.

Figure 2

Title : ATXN2 CAG repeats distribution

Footnote : Distribution of *ATXN2* CAG repeats and intermediate alleles in patients and controls

Figure 3

Title : ATXN1 CAG repeats distribution

Footnote : Distribution of *ATXN1* CAG repeats and intermediate alleles in patients and controls

Figure 4

Title : Overview of the results

Footnote The different diagnostic groups are represented in balloons under the most common proteinopathies known to underlie these diseases. Outline rings represent higher frequency of IAs compared to controls