

LETTER TO THE EDITOR

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Interrupted CAG expansions in *ATXN2* gene expand the genetic spectrum of frontotemporal dementias

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Spinocerebellar ataxia type 2 (SCA2) is due to a CAG repeat expansion in *Ataxin-2* gene (*ATXN2*), encoding a polyglutamine (polyQ) stretch. Thirty-four or more uninterrupted (pure) CAG repeats are associated with cerebellar ataxia, slow saccades, and parkinsonism, beginning before 60 years [1]. SCA2 is associated with neuronal loss in the cerebellum, *substantia nigra*, striatum and globus pallidus; and intranuclear aggregation of polyglutamine stretches, labelled by 1C2 antibody, in the cerebellum [2]. When interrupted by CAA motifs, full CAG expansions produce isolated levodopa-responsive parkinsonism [3]. On the other hand, intermediate alleles greater than 26 [4], and up to 39 CAG repeats [5], represent a strong risk factor for amyotrophic lateral sclerosis (ALS) associated with neuronal TDP-43 (TAR DNA binding Protein 43) inclusions.

ATXN2 contribution to TDP-43-proteinopathies has been studied mostly in ALS. We evaluated the contribution of *ATXN2* in 31 patients with frontotemporal lobar degeneration (FTLD) and pathologically proven TDP-43 inclusions (FTLD-TDP) without known related mutation (Supplementary methods, Additional file 1: Table S1). One patient (patient 5, Additional file 1: Table S1)

carried a 39 CAG expansion interrupted by four CAA motifs (CAG₈-CAA-CAG₄-CAA-CAG₄-CAA-CAG₉-CAA-CAG₁₀), and a 27 CAG intermediate allele (CAG₈-CAA-CAG₄-CAA-CAG₄-CAA-CAG₈) (Supplementary results). The patient developed agrammatism, word omissions and dysarthria, suggestive of nonfluent primary progressive aphasia, at age 70 (Supplementary results). Brain imaging revealed frontal, left peri-sylvian and parietal atrophy; cerebellum was normal (Fig. 1 a-d). At age 73, the association of marked frontal executive dysfunction (planning, attention, inhibition, mental flexibility deficits), ideomotor apraxia (praxis score: 4/23), akinetic-rigid parkinsonism, with asymmetric fronto-temporo-parietal atrophy, was consistent with a frontal-behavioral subtype of corticobasal syndrome (CBS) [6]. He had no cerebellar syndrome. He died at age 77. No information about the patient's family was available.

A post-mortem examination was performed (Supplementary methods and results). Both 39 and 27 CAG alleles were found in all studied brain structures (frontal cortex, striatum, mesencephalon, occipital cortex, cerebellum) (Additional file 1: Figure S1 and Figure S2). Macroscopic examination revealed marked atrophy of frontal, temporal lobes, Ammon's horn (CA1) and the subiculum. Neuronal loss and gliosis, associated with a superficial laminar spongiosis, were severe in the superficial layers of the middle frontal gyrus, motor cortex, supramarginal gyrus, CA1 and the subiculum. The pons (including the *locus coeruleus*), the cerebellum (Fig. 1e) and the dentate nucleus were normal. TDP-43,

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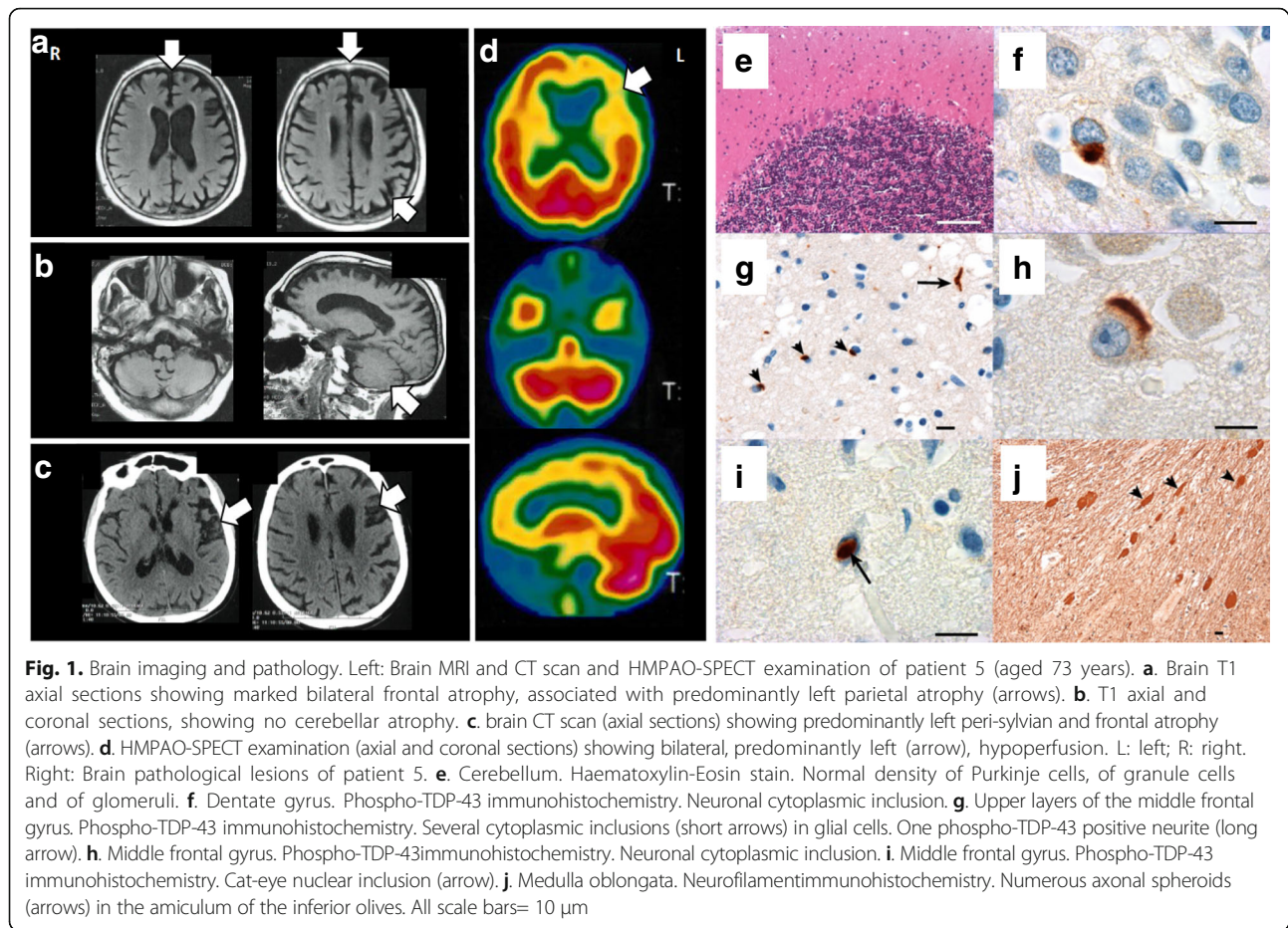
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pTDP-43, p62 and ubiquitin immunohistochemistry revealed small round cytoplasmic inclusions, sometimes glial, more abundant in the superficial layers of the middle frontal gyrus, motor cortex, and supramarginal gyrus (Fig. 1e-j). Rare pTDP-43 ‘cat eye’ intranuclear inclusions were detected (Fig. 1i). The presence of TDP-43 positive cytoplasmic inclusions, mainly distributed in the upper layers of the cortex, lead to the diagnosis of type A FTLTDP [7]. Few cytoplasmic inclusions were found in the dentate gyrus (Fig. 1f). Scarce TDP-43 and pTDP43 positive neurites were present, mainly in the frontal cortex and the supramarginal gyrus (Fig. 1g-i). No skein like inclusions were observed in the hypoglossal nucleus. Ubiquitin and p62 immunohistochemistry did not reveal inclusion in the cerebellum. No intranuclear inclusions were detected with 1C2 antibodies. No alpha-synuclein immunostaining was noted in the *substantia nigra*. Ataxin2 immunohistochemistry revealed granular cytoplasmic staining in Purkinje cells of the cerebellum and in neurons of spinal cord, similar to that found in an ALS case with intermediate 32 CAG expansion, and weak diffuse cytoplasmic staining in some neurons of the frontal cortex (Additional file 1: Figure S3).

The presentation by CBS, without cerebellar ataxia, or cerebellum lesions on imaging and pathological examination (Fig. 1b, e), was clearly distinct from SCA2 phenotype. The neuropathology, characterized by pTDP-43-positive inclusions in the neocortex, but no cerebellar lesions or 1C2 inclusions, was also different from SCA2 patients (Additional file 1: Table S2). The phenotype was distinct from the late levodopa-responsive parkinsonism associated with interrupted expansions [3, 8]. Lastly, Lewy bodies and 1C2-positive inclusions in *substantia nigra*, pontine nuclei and cerebellum, described in few parkinsonian patients [9], were absent in our case. This study shows that *ATXN2* phenotypes are not restricted to cerebellar ataxia, parkinsonism and ALS, but are expanded to pure isolated FTLTDP phenotypes. Although a coincidental occurrence of FTLTDP and *ATXN2* mutation cannot be formally excluded, all known FTLTDP and ALS genes were normal in our patient. More importantly, an interrupted *ATXN2* expansion was previously identified in a patient with FTD-ALS and type B FTLTDP pathology [10], both cases thus strongly support the causative genetic link between FTLTDP and *ATXN2*.

The clinical variability of *ATXN2* expansions is not fully explained. In most repeat expansion disorders,

somatic mosaicism of the expanded alleles contributes to the clinical expression. Conversely to pure *ATXN2* expansions, no (or a low level of) brain mosaicism was observed in this case, probably because interrupted expansions are more stable than pure ones. Interrupted expansions are also more stable than uninterrupted ones across meiosis [3]. It possibly confers a risk of anticipation lower than in pure CAG repeats, which should be considered in genetic counselling. Interrupted and pure expansions may have selective topographic toxicity involving preferentially the subcortical or the neocortical structures, causing either isolated parkinsonism or cortical syndromes. Distinct composition and/or localization of CAAs within interrupted expansions could, in turn, be associated with different patterns of neurodegeneration. Finally, the phenotype might be directly impacted by the modification of RNA secondary structure of *ATXN2* transcripts including one or more CAA. CAA motif interruptions decrease the stability of CAG hairpin as indicated by higher ΔG in predicted secondary structure (Additional file 1: Figure S2). These differences can influence the set of RNA-binding proteins interacting with *ATXN2* RNAs, and possibly interfere with RNA processing, localization or translation in specific brain structures [11].

Our patient's peculiar phenotype could be related to the 27 intermediate allele, which composition is similar that of ALS patients carrying 27 CAG alleles [12]. However he did not have ALS symptoms, and we could not evidence selective loss of Purkinje cells in cerebellar vermis [13], motor neurons alterations, nor cytoplasmic filamentous pTDP-43 inclusions in motor cortex and brainstem characteristic of ALS with intermediate alleles [13, 14]. As such, our patient's phenotype is more likely related to the 39 repeat expansion.

Finally, our patient showed a rather unique lesional pattern, characterized by FTLD-TDP type A, distinct both from pure or interrupted expansions and intermediate alleles, that expands neuropathological hallmarks associated with *ATXN2* expansions (Additional file 1: Table S3). TDP-43 inclusions were described in one patient carrying 42 pure *ATXN2* expansion carrier [15], as well as in SCA3, SCA7 and Huntington's diseases, three other CAG expansion disorders. Together, these studies and ours provide robust arguments that common TDP-43 related pathways can be involved, not only in FTLD and ALS, but also in several CAG expansion disorders including interrupted *ATXN2* expansion disease. Based on the present study, it is difficult to assert how interrupted or pure expansions modify the cellular localization of TDP-43 in neurons. However, it has been evidenced that *ATXN2* protein ortholog associates with TDP-43, induces its mislocalization and modifies its toxicity in yeast and drosophila models [4].

In summary, this case sheds new light on the significance of *ATXN2* in the spectrum of FTLD and TDP-43 pathologies and raises new challenges in the strategy that has to be applied to reach the molecular diagnosis of FTLD. It enlarges the mutation spectrum of isolated FTLD, showing that *ATXN2* should be analyzed in FTLD patients, or more largely in TDP-43 cases without known FTLD mutations, even in absence of personal or familial history of cerebellar ataxia, ALS or parkinsonism.

Additional file

Additional file 1: Supplementary methods, cohorts description, molecular analyses and immunostaining. (DOCX 17641 kb)

Abbreviations

ALS: Amyotrophic Lateral Sclerosis; *ATXN2*: Ataxin-2; CBS: CorticoBasal Syndrome; CT scan: Computed Tomography scan; FTLD: FrontoTemporal Lobar Degeneration; MRI: Magnetic Resonance Imaging; PolyQ: Polyglutamine; RNA: Ribonucleic Acid; SCA2: SpinoCerebellar Ataxia type 2; SCA3: SpinoCerebellar Ataxia type 3; SCA7: SpinoCerebellar Ataxia type 7; SPECT: Single Photon Emission Computed Tomography; TDP-43: TAR DNA binding Protein 43; pTDP-43: Phospho-TAR DNA binding Protein 43

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Availability of data and material

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Authors contributions

CD, DS, CF, VS, LMP: pathologic assessments; CF, AC, VA, MB: molecular analyses; CD, DS, VS, ST, LMP: brain bank coordination, preparation and delivery of tissues; SSB, RVS: clinical evaluation of the patients; CF, VA, CD, ILB: redaction of the manuscript; AB, ML, GS: critical revisions of the manuscript; ILB, CD: supervision of the study. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All tissue samples were obtained according to French and Spanish Legislation with transfer authorization. The informed consent for *post-mortem* and genetic studies were signed by the patients or by their legal

representatives in their name, as approved by local ethics committees and allowed by Spanish and French law and by the French Ministry of Social Affairs and Health (AC-2013-1887, DC2015-2566, AC 2015-2576). Consent for publication of the results have been obtained.

Competing interests

The authors declare that they have no competing interests

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