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BRIEF COMMUNICATION

Analysis of *HTT* CAG repeat expansion in Italian patients with amyotrophic lateral sclerosis

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Abstract

HTT full-penetrance pathogenic repeat expansions, the genetic cause of Huntington's disease (HD), have been recently reported in a minority of frontotemporal dementia/amyotrophic lateral sclerosis (ALS) patients (0.13%). We analyzed *HTT* CAG repeats in an Italian cohort of ALS patients (n = 467) by repeat-primed polymerase chain reaction. One patient harbored two expanded alleles in the *HTT* gene (42 and 37 CAG repeats). The absence of HD typical symptoms and the clinical picture consistent with ALS, corroborated by the diagnostic assessment, apparently excluded a misdiagnosis of HD.

Introduction

Dewan and colleagues have recently reported *HTT* full-penetrance pathogenic repeat expansions in three probands (0.12%) out of 2442 frontotemporal dementia (FTD)/amyotrophic lateral sclerosis (ALS), patients.[1] After expanding the analysis to an independent cohort of 3674 FTD/ALS patients, five additional carriers of *HTT* pathogenic expansions were identified (0.14%). Comparing these data to the prevalence of pathogenic *HTT* repeat expansions in the general population (0.03%),[2,3] the authors concluded that the carrier rate was significantly higher in FTD/ALS patients.

Thomas and colleagues have recently challenged this finding, highlighting several points which argue against the role of *HTT* pathogenic expansions in FTD/ALS.[4] Among them, the authors cited a previously published work which reported a 0.18% carrier rate of *HTT* repeat expansions in the general population. Accordingly, they suggested that the occurrence of *HTT* pathogenic expansions in FTD/ALS might merely reflect their prevalence among the general population.[5] Furthermore, Thomas and colleagues questioned the lack of clinical description of the cases. Indeed, they could have been misdiagnosed due to the clinical heterogeneity of HD, especially in juvenile forms, and to the age-dependent penetrance of *HTT* pathogenic expansions.[6,7] Regarding neuropathology, Thion and coauthors stated that the absence of neostriatal atrophy was coherent with the small repeat expansions of the investigated patients, and that the co-existence of huntingtin and TAR DNA-binding protein 43 (TDP-43) aggregates and motor neuron loss had been previously reported in HD brains.[8,9]

In this scenario, we performed a screening analysis of CAG repeats in *HTT* in our cohort of ALS patients, to

further assess huntingtin role in motor neuron disorders (MNDs).

Methods

A screening analysis of *HTT* CAG repeats was conducted by repeat-primed polymerase chain reaction (RP-PCR) in a cohort of Italian patients, who were diagnosed with ALS according to the El Escorial criteria,[10] as previously described.[11] A group of 298 Italian healthy subjects (aged >60 years) was used as control group. The length of expanded alleles was estimated by RP-PCR and confirmed by fluorescent PCR. Sanger sequencing was used to exclude repeat interruptions and modifications in the polyproline sequence following CAG repeats. Carriers of expanded *HTT* alleles underwent a targeted next-generation sequencing (NGS) panel to analyze genes implicated in MNDs (Table S1). The library was generated by using a 150-bp amplicon-based approach (Haloplex, Agilent) and sequenced on MiSeq instrument (Illumina). Reads were aligned to the human genome (assembly hg19), and the identified variants were annotated (ANNOVAR) and filtered, focusing on rare variants (≤0.5% in public databases), causing changes potentially damaging for the protein function.

Results

Within our cohort (n = 467), ALS patients' mean age at onset was 60.6 years (SD 13.7), and 60.9% were males. Familial cases were 6.6%. The distribution of CAG repeats in the ALS cohort was evaluated and compared to a control group of Italian healthy subjects (n = 298, age at sampling >60 years) (Fig. 1). The difference observed between the two groups failed to reach statistical significance (Mann–Whitney U-test, p = 0.056). The repeat frequency of normal (CAG < 27) and unstable intermediate (27 < CAG ≤ 35) alleles was not different in the two groups (Fisher's exact test, p > 0.05). The proportion of intermediate alleles was identical in the control (14/298 = 4.7%) and in the ALS (22/267 = 4.7%) groups and comparable to that observed in an independent cohort of Italian control subjects (5.3%).[12]

Interestingly, we identified one ALS patient who harbored two expanded alleles in the *HTT* gene with 42 and

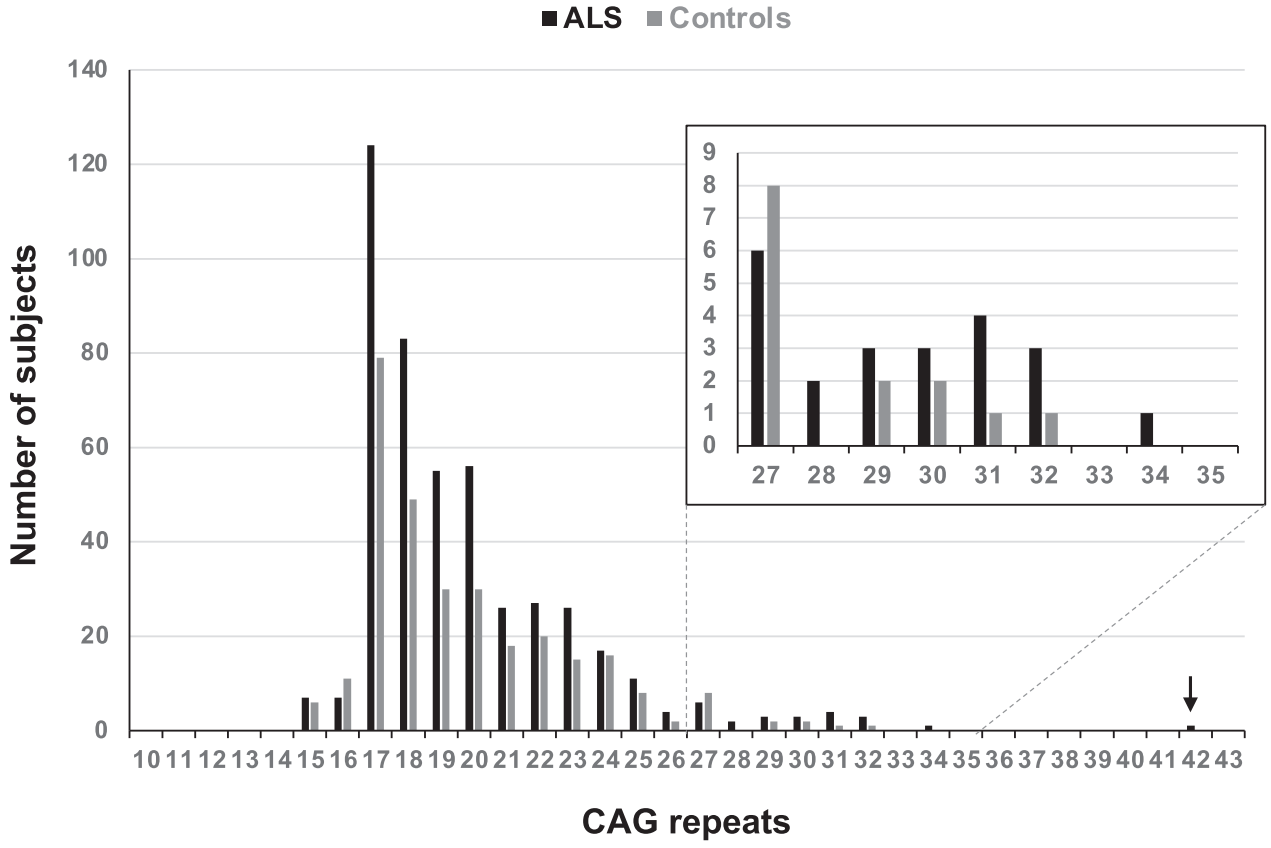

Figure 1. *HTT* CAG repeat length (largest allele) in 466 ALS patients and 292 Italian control subjects (age at sampling >60 years). The insert shows a zoomed view of *HTT* intermediate alleles (27–35 CAG repeats). The arrow indicates the ALS Patient harboring a full-penetrant pathogenic *HTT* allele described in the text.

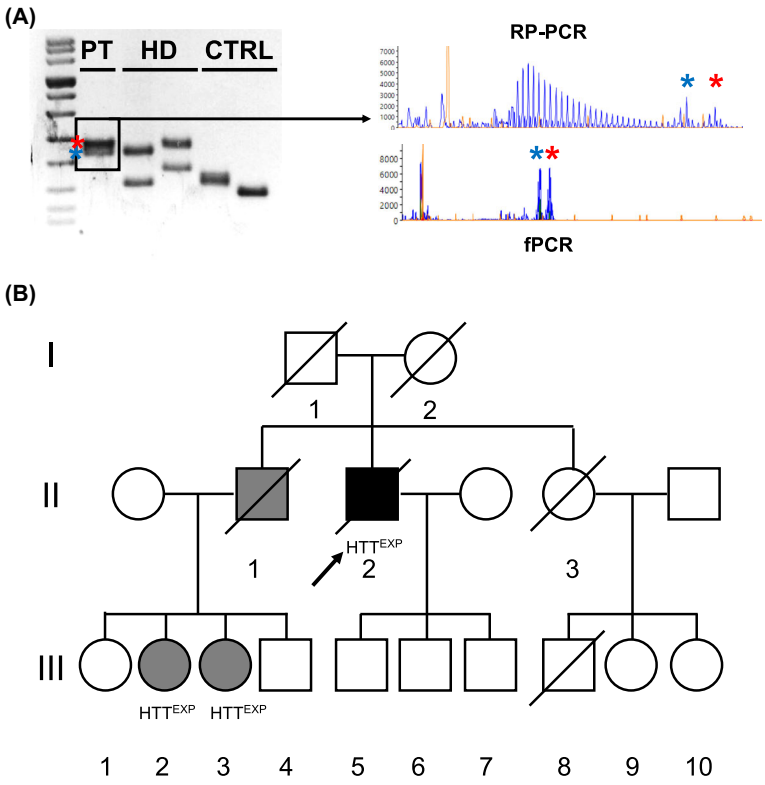

Figure 2. (A) (left) PCR amplicons encompassing *HTT* CAG repeat regions electrophoresed through agarose gel amplified starting from DNA obtained of the patient described in manuscript (PT), two HD subjects (size of the largest alleles: 38 and 42, respectively) and two control subjects. (right) Electropherograms obtained from patient's DNA after repeat-primed (RP-PCR) and fluorescent (fPCR) PCR amplification, displaying the saw-tooth pattern and the expanded *HTT* alleles (37 CAG in blue, 42 CAG in red). (B) Pedigree of the family described in the text. Roman and Arab numbers are used to indicate the generation and the subject within each generation. The arrow indicates our proband. Black and gray symbols indicate disease status (clinical diagnosis of ALS and HD, respectively). HTT^{exp} indicates a positive molecular testing for CAG repeats expanded HTT alleles.

37 CAG repeats (Fig. 2A and Fig. S1). By performing targeted NGS panel analysis, no additional disease-causing mutations were detected in genes implicated in MNDs in this patient (Subject II-2 in Fig. 2B). The hexanucleotide pathogenic expansion in *chromosome 9 open reading frame 72* (*C9ORF72*) was also excluded.

At the age of 61, the patient started complaining of muscle weakness in the upper limbs, mainly on the right. Neurological evaluation showed signs of muscle wasting and asymmetric brisk deep tendon reflexes at the upper limbs. Electromyography detected chronic neurogenic changes and acute denervation signs in the distal muscles of both upper limbs. Brain and spinal cord magnetic resonance imaging (MRI) ruled out alternative diseases, and a diagnosis of possible ALS was formulated. Eighteen months later, he developed progressive dysphagia and severe respiratory impairment. Therefore, percutaneous endoscopic gastrostomy (PEG) and tracheostomy were placed. No cognitive impairment nor psychiatric disturbances were reported. He died of respiratory insufficiency at 64. The proband was born to Italian, nonconsanguineous parents. The father died at 35 years during the Second World War. The mother was asymptomatic for neurological disorders and died at 80 years of age. The patient had two siblings. His sister (II-3) died at 60 years of lung carcinoma, with no signs or symptoms of nervous system dysfunction. She had one son (III-8), who died in a car accident at 30 years, and two currently healthy daughters, of 62 and 60 (III-9, III-10). The proband's brother (II-1) died at 70 years of age. At 55, he had been diagnosed with HD. One daughter (III-1) and the only son (III-4) of, respectively, 67 and 50 years have not been tested. She is asymptomatic for neurological or psychiatric disorders, while he recently developed psychiatric symptoms. The remaining two daughters, currently of 61 and 59 years of age, carry full-penetrance pathogenic repeat expansions in *HTT*. The older daughter (III-2) developed psychiatric symptoms at 35 years of age, choreic movements, and extrapyramidal motor signs at 50, and she later developed cognitive impairment. Genetic testing revealed 42 and 18 CAG repeats in *HTT* alleles. The younger daughter (III-3) developed depression at age

45, followed by choreic movements. Our proband has three sons, respectively, 48, 42, and 36 years old (III-5, III-6, III-7). They have not shown signs of neurological involvement so far, and they refused to pursue the predictive genetic testing.

Discussion

In our case, the positive family history for HD and the absence of MND in the relatives who harbor a pathogenic *HTT* repeat expansion might suggest a "double-trouble" in the proband. Our patient could be affected, at least apparently, by sporadic, rapidly progressive ALS, which led to death before the appearance of HD signs or symptoms. Although we ruled out molecular defects in a large panel of genes involved in MNDs (including all the ALS causative genes), an undisclosed genetic or environmental predisposing factor might have increased the susceptibility to develop a MND phenotype. The absence of HD in our proband might be explained by the wide age at onset variation observed among HD patients with CAG repeats between 40 and 42. Indeed, the age at onset of Italian HD patients with 42 CAG repeats is 39–69 years.[13]

However, the remarkable difference of age at onset within the family (about 20 years) and the absence of psychiatric or motor symptoms consistent with HD in our proband, who reached the seventh decade, deserve further reflections.

Our proband harbored two expanded *HTT* alleles. Subjects harboring two *HTT* expanded alleles are rare with estimated prevalence ranging from 0.1%[14] to 0.4%[15] within HD cohorts. The largest *HTT* allele, usually considered the main determinant for disease onset and progression, is likely shared by the patient's brother and nieces. The minor allele (37 repeats) confers a low-penetrance risk for HD and could potentially be involved in modifying the clinical presentation. Indeed, *HTT* alleles with 37 repeats are considered pathogenic with reduced penetrance ($37 < CAG < 40$). Nevertheless, biallelic HD patients do not display significant differences in disease onset or progression compared to those harboring a single mutated allele,[16] supporting the "complete dominance" of HD.

Several reports have described the occurrence of ALS in patients with a family history of HD and a positive genetic testing for high-penetrance pathogenic CAG repeat expansion in *HTT*.[17–20] Interestingly, in two cases the available *post-mortem* tissues displayed concomitant ALS and HD pathology,[21,22] a finding recently paralleled in larger independent HD brain cohorts.[18]

These findings suggest that *HTT* expansion might predispose a subset of individuals to develop clinical and pathological features consistent with ALS. This hypothesis cannot be definitively excluded in our patient. Considering the clinical picture consistent with MND, corroborated by the diagnostic assessment, and the absence of HD typical symptoms, we exclude a misdiagnosis of HD at the time of clinical evaluation of our patient. The presence of a positive family history for HD differentiates our case from those described by Dewan and colleagues.[1]

Our study indicates that CAG abnormal repeats in *HTT* might be extremely rare in Italian ALS patients. The absence of pathological *HTT* repeat expansions in the remaining cohort might have been influenced by its demographic features, especially by the low rate of ALS familial cases (6.6%). Indeed, out of eight carriers of abnormal *HTT* CAG repeats reported by Dewan and colleagues, four had a known family history of FTD/ALS. This finding suggests that *HTT* pathogenic expansions might be more common in familial rather than sporadic FTD/ALS.[1] We did not observe a higher rate of intermediate (27–35) *HTT* repeat expansions in ALS patients compared to control subjects, although the difference in the distribution of CAG repeats between these two groups was nearly significant.

The association of expanded *HTT* alleles with heterogeneous clinical presentations might acknowledge the variable expressivity of this molecular defect. Otherwise, it might indicate that clinically distinct neurodegenerative disorders such as ALS and HD could share a common genetic basis. A similar hypothesis has been postulated for other repeat expansion disorders. Hexanucleotide repeat expansions in *C9ORF72*, the main genetic cause of ALS/FTD, have been linked to different neurodegenerative disorders, including dementia and parkinsonism,[23] and represent the most common genetic cause of HD phenocopies.[24] Notably, pathological CAG expansions in *HTT* can also induce TDP-43 and huntingtin co-aggregation in vitro, likely through a direct interaction between poly-Q residues and TDP-43 C-terminal domain.[8] Therefore, *HTT* expansion could represent an additional example of the pathogenic link connecting poly-Q expansions and TDP-43 proteinopathies, including ALS/FTD.

In summary, we believe that the direct involvement of *HTT* in ALS pathogenesis, emerged by novel sequencing technologies, should be interpreted with great prudence. Although it is possible that HD and ALS are part of the same neurodegenerative disease landscape and that *HTT* expansion might produce ALS features as the primary manifestation in a small minority of HD individuals, available data so far are limited and fragmented. A replication of these studies in further cohorts is required to confidently confirm the pathogenic role of *HTT* repeat expansions in ALS before moving to common clinical practice.

Author Contributions

AM: conceptualization; data curation; formal analysis; investigation; writing–original draft preparation; writing–review and editing. DG: conceptualization; data curation; writing–original draft preparation; writing–review and editing. MM: writing–review and editing. SA: investigation. RDB: writing–review and editing. CS: writing–review and editing. GPC: writing–review and editing. SC: writing–review and editing. DR: conceptualization; data curation; formal analysis; investigation; writing–review and editing.

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Conflict of Interest

RDB, GPC, and SC are members of the FALS Sequencing Consortium. The other authors declare no existing conflict of interests.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Sanger sequencing chromatograms of the *HTT* CAG repeat expansion. *HTT* CAG repeat length in the patient described was assessed by cloning followed by Sanger sequencing. Electropherograms showing the two alleles containing 37 and 42 CAG repeats, respectively.
Table S1. List of genes included in our NGS panel.