Molecular Chaperones in the Pathogenesis of Amyotrophic Lateral Sclerosis: The Role of HSPB1

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ABSTRACT: Genetic discoveries in amyotrophic lateral sclerosis (ALS) have a significant impact on deciphering molecular mechanisms of motor neuron degeneration but, despite recent advances, the etiology of most sporadic cases remains elusive. Several cellular mechanisms contribute to the motor neuron degeneration in ALS, including RNA metabolism, cellular interactions between neurons and nonneuronal cells, and seeding of misfolded protein with prion-like propagation. In this scenario, the importance of protein turnover and degradation in motor neuron homeostasis gained increased recognition. In this study, we evaluated the role of the candidate gene *HSPB1*, a molecular chaperone involved in several proteome-maintenance functions. In a cohort of 247 unrelated Italian ALS patients, we identified two variants (c.570G>C, p.Gln190His and c.610dupG, p.Ala204Glyfs*6). Functional characterization of the p.Ala204Glyfs*6 demonstrated that the mutant protein alters HSPB1 dynamic equilibrium, sequestering the wild-type protein in a stable dimer and resulting in a loss of chaperone-like activity. Our results underline the relevance of identifying rare but pathogenic variations in sporadic neurodegenerative diseases, suggesting a possible correlation between specific pathomechanisms linked to *HSPB1* mutations and the associated neurological phenotype. Our study provides additional lines of evidence to support the involvement of *HSPB1* in the pathogenesis of sporadic ALS.

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KEY WORDS: sALS; HSPB1; chaperone activity; molecular modelling

Additional Supporting Information may be found in the online version of this article.

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Introduction

Amyotrophic lateral sclerosis (ALS) is a fatal, adult onset neurodegenerative disorder characterized by the progressive loss of upper and lower motor neurons. The disease is mainly sporadic (sALS), but 5–10% of patients have a positive family history (fALS). Starting from the hypothesis of a sporadic disorder as the result of complex genome–environment interactions, the inheritability of sALS has been largely demonstrated in recent years. Although significant advances have been made in the molecular genetics of ALS, the screening of the currently known large- or small-effect genes only solves a small fraction of sporadic cases and the etiology of sALS remains mostly unexplained [Finsterer and Burgunder, 2014].

In this study, we evaluated the involvement of the *small heat-shock protein family B member 1* (*HSPB1*) (MIM#602195) in the pathogenesis of ALS. The *HSPB1* gene encodes an inducible molecular chaperone able to reduce the aggregation of misfolded proteins [Haslbeck et al., 2015]. HSPB1 has been found to be upregulated in ALS mouse models [Vleminckx et al., 2002], as well as in the lumbar spinal cord specimens of ALS patients [Anagnostou et al., 2010]. In addition, mutations in this gene result in autosomal dominant or recessive axonal peripheral neuropathies (distal hereditary motor neuropathy [dHMN] and Charcot-Marie-Tooth [CMT] type 2F), highlighting its relevance in maintaining neuronal integrity [Evgrafov et al., 2004; Houlden et al., 2008]. Here, we report the identification of two patient-specific *HSPB1* mutations in two sporadic Italian ALS patients, additionally confirming its possible role in the pathogenesis of this disorder.

Materials and Methods

Cohort Enrolment and Genetic Studies

The selected cohort was composed of 247 unrelated Italian patients presenting a clinical diagnosis of ALS. A first group of patients (*n* = 153) has been enrolled in frame of the multicenter-multisource prospective population-based registry "Liguria Amyotrophic Lateral Sclerosis Registry" [Bandettini di Poggio et al., 2013] and through the collaboration of other Italian centers. The second group of patients (*n* = 94) has been enrolled at the Turin ALS center.

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Patients underwent neurological examination and were diagnosed according to the El-Escorial revised criteria [Brooks et al., 2000]. The description of the enrolled cohort is provided in Supp. Table S1. Informed consent was obtained from all participants and the study was approved by the local ethics committee. The cohort was selected based on the exclusion of pathogenetic variants in the major genes associated with ALS pathogenesis (*SOD1*, *TARDBP*, *FUS*, and *C9orf72*).

The coding regions of *HSPB1* (Ensembl reference sequence: ENST00000248553) was analyzed by direct sequencing (*n* = 153) or denaturing high-performance liquid chromatography (DHPLC) (Wave MD–Transgenomics, New Haven, CT, USA), followed by Sanger sequencing of the variant chromatograms (*n* = 94). The SNP rs545738637 T>C previously identified in *HSPB1* promoter [Dierick et al., 2007] was tested by direct sequencing at the genomic position 7:76302496 (GRCh38). Sequencing products were run on a 3130xl Genetic Analyzer (Applied Biosystems–Thermofisher, Waltham, MA, USA) and analyzed with SeqScape Software v.2.7 (Applied Biosystems–Thermofisher, Waltham, MA, USA). For cDNA numbering, nucleotide numbering uses +1 as the A of the ATG translation initiation codon in the reference sequence, with the initiation codon as codon 1. The ethnically matched control cohort (*n* = 250) was analyzed by DHPLC (Wave MD–Transgenomics, New Haven, CT, USA). Whole-exome sequencing of patient *ALS-160* was performed by deCODE Genetics, Iceland. Exome enrichment was performed using Nextera and run on HiSeq2500 (Illumina, San Diego, CA, USA). Reads alignment and variants calling was performed with BWA and GATK software. The methods used for the functional studies are detailed in the Supporting Information Material.

Molecular Modeling

Up to now, various crystallographic data of human heat-shock proteins (HSPs) have been obtained and released on protein data bank, one of the latest being human HSPB1 α-crystallin domain (ACD; 84–176 residues) (pdb code: 4MJH; resolution = 2.60 Å) [Hochberg et al., 2014]. Since small HSPs share a highly conserved ACD, a whole human B1 theoretical model was performed by homology modeling techniques, on the basis of a multiple template alignment strategy focused on the X-ray data of the available HSPs, as recently published [Fossa and Cichero 2015]. The HSPB1 mutant models were obtained by the residue rotamer explorer implemented in the MOE sequence editor (Chemical Computing Group Inc., Montreal, Canada), followed by residue minimization using AMBER99 force field. Aggregation-prone regions in the three models were calculated using the Protein Patch Analyzer module implemented in the MOE software.

Results

Genetic Findings

The molecular analysis of *HSPB1* was performed in both the patient (*n* = 247) and the control (*n* = 250) cohort. The genetic screening revealed the presence of two patient-specific variations (c.570G>C, p.Gln190His and c.610dupG, p.Ala204Glyfs*6; variations submitted in http://www.lovd.nl/HSPB1), together with additional synonymous variations (Fig. 1A).

The heterozygous missense mutation c.570G>C, p.Gln190His (Fig. 1B, left) was identified in the sporadic ALS patient *SLA2014-469*. The residue p.Gln190 is conserved among HSPB1 orthologous (Fig. 1D) and *in silico* predictions using SIFT and Polyphen-2 show the missense variant to be deleterious with the score of 0.00 and

0.974, respectively (see details under the Supporting Information Material).

The heterozygous frameshift c.610dupG, p.Ala204Glyfs*6 (Fig. 1B, right) was identified in the sALS patient *ALS160*. The guanine duplication affects the third to last codon of *HSPB1* and leads to the translation of eight additional base pairs in the 3′UTR of the gene, resulting in an extended mutant protein (Fig. 1C). The alignment of HSPB1 C-terminal domain across different species showed the conservation of the p.Ala204 amino acid and none of the selected HSPB1 orthologous displayed a similar C-terminal extension (Fig. 1D).

The variants c.570G>C and c.610dupG were absent from public databases for human variations (dbSNP138, 1000 Genomes, Exome Variant Server and ExAc v.03) and were not identified in the selected control cohort. Moreover, in order to exclude the association of these variants with an inherited peripheral neuropathy phenotype, their presence was excluded by screening an additional 660 chromosomes of dHMN or CMT patients. The occurrence of other possible pathogenic mutations in the patient *ALS160* has been excluded by whole-exome sequencing, after filtering against the full up-to-date list of ALS-associated genes. Interestingly, the promoter variant rs545738637 T>C previous identified in a Belgian sALS patient was not identified in our cohort.

Clinical Description of the Patients Harboring *HSPB1* Mutations

Case 1: *SLA2014-469 (p.Gln190His)*. The patient *SLA2014-469* developed dysphagia and dysarthria at the age of 58 years. Few months later, she showed weakness and atrophy at the muscles of the right hand. Neurophysiological examination showed active and chronic denervation at upper and lower limbs and at genioglossus muscle. Spirometry showed a marked restrictive pattern (FVC 56%) and creatine kinase was increased. Neuropsychological examination showed a mild cognitive impairment, mainly behavioral. Although her family history was negative for ALS, her mother was referred as affected by Alzheimer's disease. She was diagnosed with definite ALS and she died from respiratory failure, 24 months after the onset.

Case 2: *ALS160 (p.Ala204Glyfs*6)*. The patient ALS160, with a negative family history for neurological disorders, at the age of 73 developed balance disturbances with frequent falls and signs of extrapyramidal involvement for which a diagnosis of possible multiple system atrophy with cerebellar predominance was made. She arrived at our attention at the age of 77 because of bulbar symptoms and diffuse signs of spinal amyotrophy. She did not show any cerebellar sign. The neurophysiological study showed axonal motor neuropathy in lower limbs and signs of denervation and reinnervation at four limbs and paravertebral muscles. A diagnosis of ALS-plus syndrome with extrapyramidal features [McCluskey et al., 2014; Pupillo et al., 2015] was made. At the age of 78, she developed scanning dysarthria, mixed dysphagia, and respiratory insufficiency leading to nocturnal noninvasive ventilation. To date, at the age of 82, neurological examination shows severe cranial involvement, diffuse hyposthenia with muscular hypotrophy, fasciculation, and brisk deep tendon reflexes at four limbs. Hypomimia with resting, postural, and kinetic tremor in both hand and head is also evident. Postural changes are performed with assistance and the patient is wheelchair bounded. Signs of cognitive impairment are not evident.

Functional Analysis

To better frame the implications of HSPB1 mutations in the pathogenesis of ALS, we studied the functional effect of the
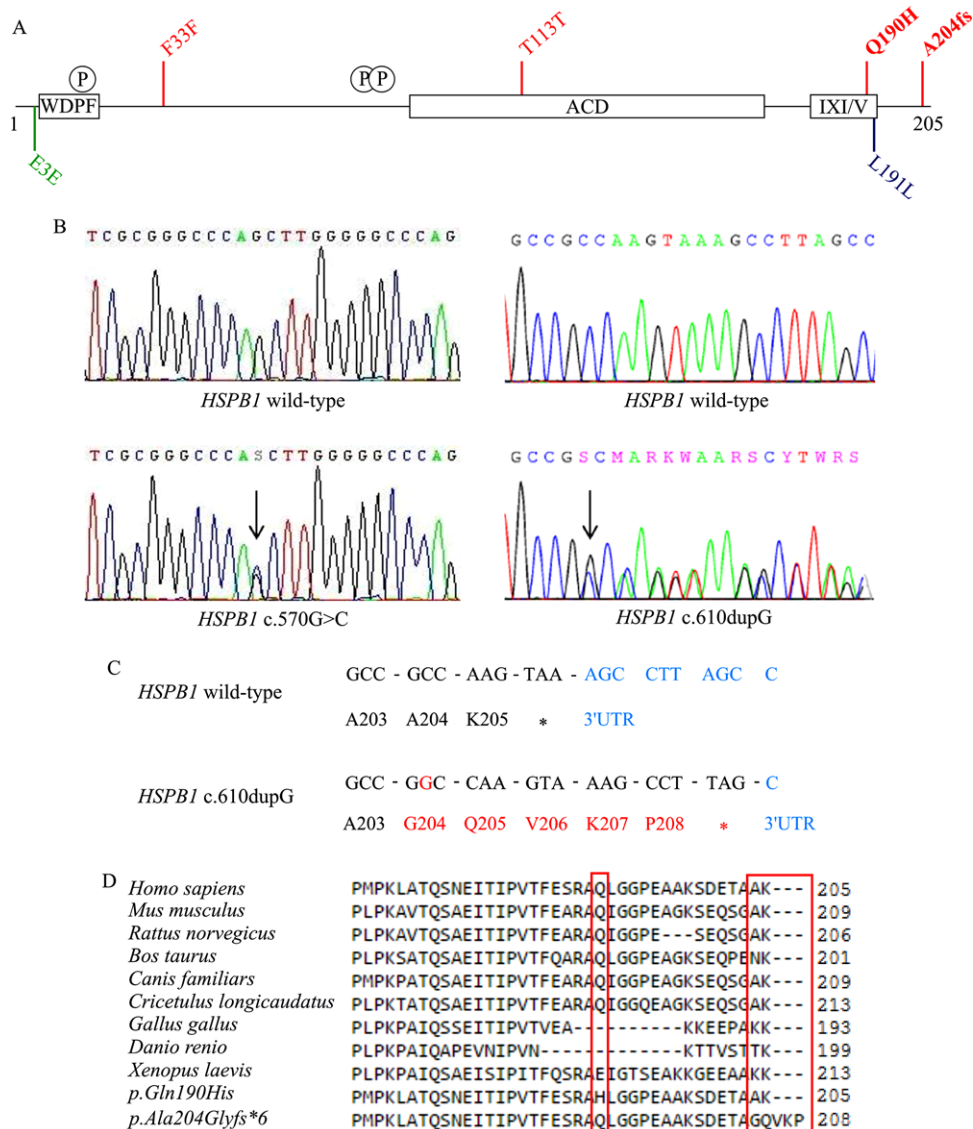
Figure 1. Molecular analysis of *HSPB1*. The patient-specific, control-specific, and shared HSPB1 variations identified in this study are depicted in red, blue, and green, respectively, on the schematic protein representation. The known HSPB1 functional domains (N-terminal motif WDPF, ACD: alpha-crystallin domain, C-terminal domain IXI/V) and the three phosporylation sites (P) on p.Ser15, p.Ser78 and p.Ser82 are highlighted (panel A). The electropherogram of the heterozygous missense mutation c.570G>C on genomic DNA is shown compared to a wild-type sequence (panel B, right). The heterozygous frameshift c.610dupG is depicted with the corresponding nucleotide difference between wild-type and mutant allele in the electropherogram data (panel B, left). The duplication of a guanine in position c.610 determines the translation of eight additional base pairs in the 3′UTR of the gene, resulting in an extended protein (panel C). Multiple alignment of HSPB1 C-terminus in different orthologous shows the conservation of the residue p.Gln190 and that none of the selected species presents with an additional C-terminal segment, as is the case for the mutant p.Ala204Glyfs*6 (panel D).

p.Ala204Glyfs*6 mutation on patient-derived lymphoblastoid cell lines (LCLs). Unfortunately, no patient-derived material was available for the mutant p.Gln190His.

HSPB1 expression and induction in ALS160 EBV-transformed LCLs

To explore the effect of the p.Ala204Glyfs*6 mutant on cellular homeostasis, we first investigated whether it could impair the *HSPB1* expression level, using patient-derived LCLs, demonstrating that both wild-type and mutant alleles are expressed on mRNA and protein level (Supp. Fig. S1A and B). On protein level, HSPB1 expression seemed to be noticeably reduced in the patient *ALS160*,

compared to controls (Supp. Fig. S1C), remarkably without triggering neither the proteasomal- nor the lysosomal-dependent degradation pathways (Supp. Fig. S2).

Although it is expressed in basal conditions, HSPB1 is striking upregulated upon cellular stress, a situation in which the concentration of aggregation-prone folding intermediates increases and this protein mainly exerts its chaperone and cytoprotective functions [Oesterreich et al., 1996]. Through heat-shock experiments, we demonstrated that HSPB1 is still inducible in patient-derived LCLs (Fig. 2A). Together, these results suggest that there is no specific degradation or defect in the expression/induction of HSPB1 in *ALS160* compared to controls. We therefore decided to evaluate whether HSPB1 was still functional upon homeostasis perturbation.
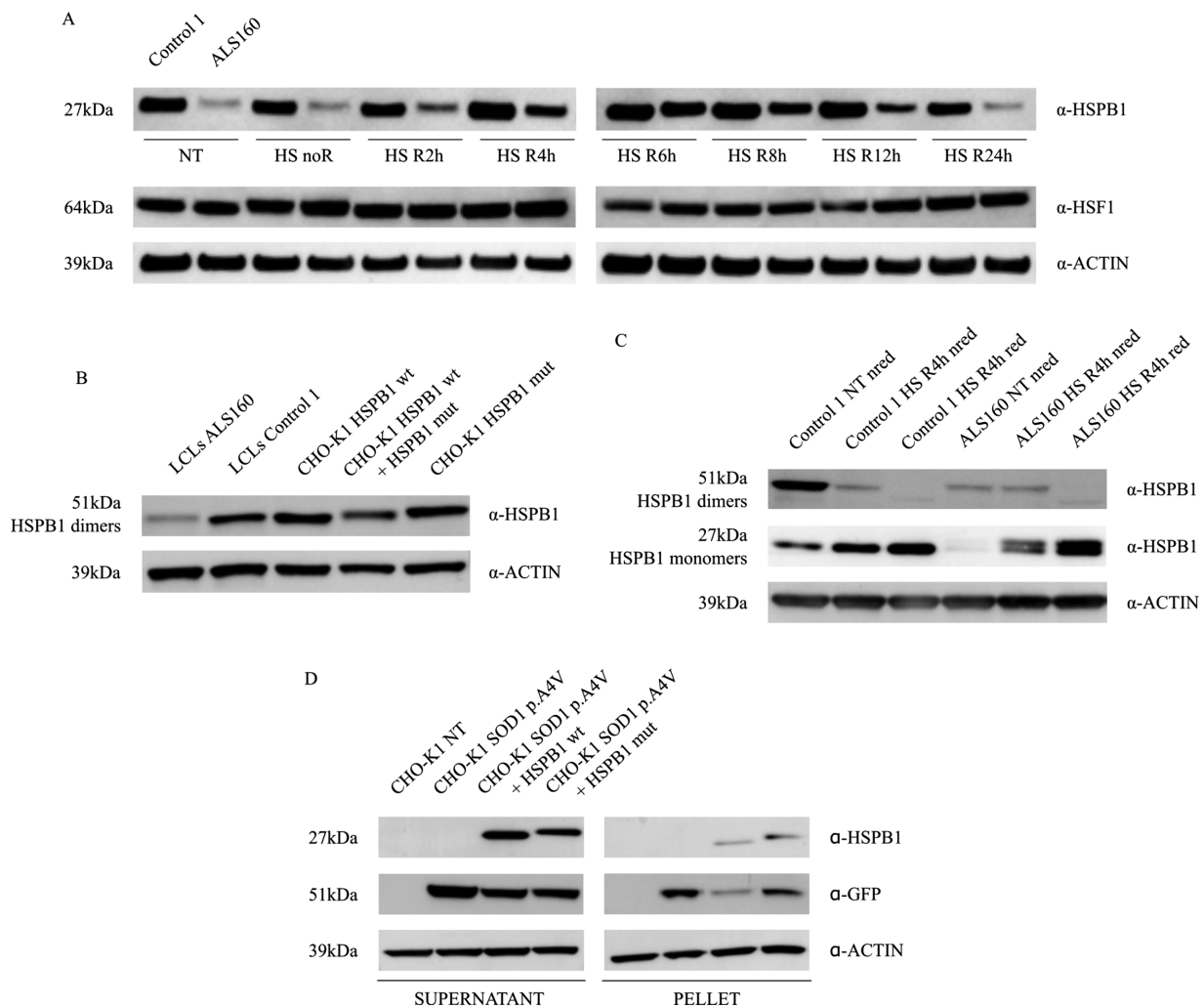
Figure 2. HSPB1 p.Ala204Glyfs*6 sequesters the wild-type protein in a stable dimer impairing its chaperone-like activity. The upregulation of HSPB1 in both control and patient LCLs upon heat shock (42°C) is depicted in panel (A). The untreated condition (NT) is compared to the heat-shock condition without recovery (HS noR) and to different recovery time points, respectively, 2, 4, 6, 8, 12 and 24 hr (HS R2h to HS R24h). As a positive control, the membranes were probed for heat-shock factor 1 (HSF1), known to be phosphorylated during heat-shock condition. The phosphorylation results in the increase in its molecular weight as depicted in panel (A) lanes 3 and 4. To evaluate the molecular weight of the dimeric structures observed in patient-derived LCLs, we compared this cell line with control LCLs and with three CHO-K1 stable cell lines expressing the constructs encoding for HSPB1 wild-type, mutant or a combination of both (panel B). The naïve CHO-K1 cell line does not express HSPB1, thus allowing evaluating its dimerization in the absence of the endogenous protein. The dimer-monomer shuttling has been evaluated in patient-derived LCLs compared to control (panel C). Basal conditions are compared to poststress conditions (HS R4h) in both nonreducing (nred) and reducing (red) settings. The chaperone-like activity of HSPB1 p.Ala204Glyfs*6 has been evaluated as its ability to clear SOD1 p.Ala4Val-GFP aggregates in double stable CHO-K1 cell lines (panel D). The clearance of SOD1 aggregates determines a different solubility of the protein, reducing the signal in the pellet fraction. HSPB1 wild-type (wt) is able to clear the SOD1 p.Ala4Val aggregates in double stable CHO-K1 cell lines as shown in lane 7. When comparing the CHO-K1 lines expressing HSPB1 wt (lane 7) to the one expressing p.Ala204Glyfs*6 (mut) (lane 8), we showed that the presence of the mutation determines an increase in the GFP signal in the pellet fraction, suggesting that the mutant protein is not able to clear the SOD1 aggregates.

HSPB1 p.Ala204Glyfs*6 sequesters the wild-type protein in a stable dimer

Small HSPs exhibit a pronounced dynamic equilibrium between monomeric, dimeric, and oligomeric state. The oligomers are believed to be reservoirs, which dissociate upon stress into the active dimer and monomer forms. The HSPB1 dimer interface is located within the alpha-crystallin domain and it is further strengthened and stabilized by the C-terminus [Vos et al., 2008]. Therefore, we evaluated whether the additional C-terminal extension of the p.Ala204Glyfs*6 mutation affected HSPB1 dimerization by analyzing the protein migration pattern in nonreducing

conditions. In the presence of two different proteins, we expected the formation of three dimer combinations (wild-type/wild-type, wild-type/mutant, and mutant/mutant). Interestingly, the patient-derived LCLs showed only one structure, with a higher molecular weight than controls. In order to better understand the mutant HSPB1 dimerization properties and composition, we used CHO-K1 cell lines, stably expressing HSPB1 wild-type, HSPB1 p.Ala204Glyfs*6, or both. Intriguingly, while both the wild-type and the mutant proteins are able to form homodimers, the double stable CHO-K1 cell line confirmed the pattern observed in patient-derived LCLs, showing only one dimer and apparently corresponding to the wild-type/mutant dimer (Fig. 2B). These data suggest that when the

two proteins are expressed together, the dimerization pattern shifts predominantly to the wild-type/mutant form.

In the context of HSPB1 dynamic equilibrium, we previously demonstrated that, during poststress recovery, the monomeric form is upregulated and the dimers dissociates into monomers [Almeida-Souza et al., 2010]. Interestingly, we demonstrated that, upon heat-shock, in patient-derived LCLs, the increased expression of monomeric HSPB1 was not associated with a dimer–monomer shuttling (Fig. 2C). This result suggests that the wild-type/mutant dimer forms a stable structure, not able to dissociate and therefore perturbing the dynamic equilibrium of HSPB1.

To explain the different dimerization properties observed, we evaluated the surface steric and electrostatic profile of the mutant C-terminus extension, building a specific 3D model. In the native HSPB1, molecular modeling data suggest that the dimers formation is promoted by the exposure of a number of weak noncovalent contacts sites in specific surface patches. While the wild-type HSPB1 displays a precise charge distribution across the C-terminal domain (Supp. Fig. S3A), the mutant protein shows marked electrostatic alterations (Supp. Fig. S3C), with a smaller highly hydrophobic region, a reduced negative charge area and an additional positive patch resulting from the mutated residues 204–208 (Supp. Table S2). Interestingly, the 3D model of the p.Gln190His revealed a clear modification of the hydrophilic properties compared with the wild-type protein (Supp. Fig. S3B), leading to a much more extended positive surface at the C-terminal domain (Supp. Table S2).

Taken together, these data suggest that the difference between native and mutant p.Ala204Glyfs*6 HSPB1 dimerization kinetics can be partially explained by different hydrophobic and polar contacts at the protein surface. In addition, while both the p.Gln190His and the p.Ala204Glyfs*6 mutations affect the charge distribution of the C-terminal domain, the p.Gln190His seems to be much more prone to aggregation rather than the p.Ala204Glyfs*6, displaying overall acceptable hydrophobic patches if compared with the native protein. However, additional functional studies are needed to confirm this hypothesis.

HSPB1 p.Ala204Glyfs*6 results in a loss of chaperone-like activity

Previous studies have shown that shuttling between monomers, dimers and oligomers is required for the proper substrate binding and chaperone-like activity of HSPB1 [Giese and Vierling, 2002]. Nonnative substrates are maintained in a folding-competent state, promoting their refolding or, when this is not possible, their degradation [Hartl et al., 2011]. Based on the different dimerization profile observed for HSPB1 p.Ala204Glyfs*6, we investigated whether this mutant HSPB1 still preserved its chaperone activity. We therefore performed an indirect clearance assay using the aggregation prone *superoxide dismutase 1* (*SOD1*) mutation p.Ala4Val as a substrate. The formation of aggregates was quantified by separating the aggregates-containing pellet fraction from the supernatant. As shown in Figure 2D, wild-type HSPB1 assists in refolding/degradation of SOD1 p.Ala4Val, reducing the amount of insoluble SOD1 p.Ala4Val in the pellet fraction. However, the presence of the HSPB1 p.Ala204Glyfs*6 increases the amount of SOD1 p.Ala4Val aggregates in the pellet fraction, when compared to the wild-type HSPB1, suggesting that the mutant protein results in a loss of chaperone-like activity.

Discussion

Several neurodegenerative disorders, as Huntington, Alzheimer, Parkinson, and ALS, are characterized by the accumulation of neuronal intracellular aggregates, which impairs neuronal connectivity and triggers cell death signaling pathways. This accumulation is caused by the incapacity of the molecular chaperones to overcome the instable folding intermediates, leading to protein insolubility and precipitation. To corroborate this hypothesis, it has been shown that the overexpression of the chaperones HSP70 and HSP90 suppresses protein aggregation, hence influencing the neuronal phenotype [Muchowski, 2002]. Interestingly, mutated SOD1 aggregates sequester a number of HSPs, including HSPB1 [Yerbury et al., 2013] suggesting that, in pathological conditions, the availability of these chaperones to undertake their housekeeping functions is drastically reduced, impairing neuronal responses to cellular stress and resulting in an increased vulnerability [Kalmar et al., 2014].

In this report, we describe for the first time two *HSPB1* coding variants identified in two unrelated sALS patients. The molecular analysis of the patient and the control cohort demonstrated that nonsynonymous variations are patient specific, as we did not identify any but synonymous variants in the control population. In addition, the incidence of frameshift variations identified in the ALS cohort analyzed in this study exceeds in respect to the data available in the ExAC database (ALS cohort: 0.405% vs. ExAC: 0.005%, *P*-value = 0.032; 95% CI, Fisher's exact test, with Bonferroni correction), while the incidence of the missense mutations is similar in both datasets, showing no statistically significant difference. The variants p.Gln190His and p.Ala204Glyfs*6 are both located in the C-terminal domain of HSPB1, a region involved in the control of its chaperone-like activity [Lelj-Garolla and Mauk, 2012]. The functional characterization of different peripheral neuropathies-related mutations, located in this domain, did not show a clear correlation between the location of the mutant and the associated cellular phenotype. While some mutations display an aggregation-prone tendency (e.g., p.Pro182Leu) [Evgrafov et al., 2004], some other located in the same proximity do not show any sign of aggregation (e.g., p.Thr180Ile) [Chalova et al., 2014]. This indicates that the nature of the substitution, instead of its location within the protein structure, determines the related cellular consequences. The availability of patient-derived material for the extended frame-shift p.Ala204Glyfs*6 allowed us to analyze the biochemical properties of this mutant, subsequently confirmed with *in vitro* studies. We demonstrated that the mutant protein sequesters the wild-type protein in a stable dimer, severely affecting HSPB1 monomer–dimer dynamic equilibrium. Although no crystal structure of the C-terminal part of HSPB1 has been deposited, the homology model of the full structure of HSPB1 has been recently described [Fossa and Cichero, 2015]. Using these coordinates, we were able to model the p.Ala204Glyfs*6 additional C-terminal extension. We confirmed that the different dimerization kinetic displayed by this mutant is sustained by an altered surface steric and electrostatic protein profile across the C-terminal domain, impairing hydrophobic and polar contact points in this area. Supporting these data, we demonstrated the p.Ala204Glyfs*6 mutation results in a loss of chaperone-like activity, being the mutant HSPB1 unable to clear aggregating substrates. The *HSPB1* gene is one of the most frequent causes of dHMN and axonal CMT disease with predominant motor involvement [Houlden et al., 2008; Capponi et al., 2011]. Functional studies on some of the CMT-associated missense mutations showed a gain of toxic function mechanism. The mutant proteins displayed hyperactivity, caused by an increased monomerization of HSPB1 and leading to the enhanced affinity to their client proteins [Almeida-Souza et al., 2010]. In particular, these mutants showed a strong binding to tubulin and microtubules, leading to the overstabilization of the microtubules network [Almeida-Souza et al., 2011]. Recent studies on the C-terminal truncated p.Met169Cysfs*2

mutation demonstrated that this terminal part of HSPB1 is necessary for cell survival upon misfolded protein-induced stress. The truncated HSPB1 mutation exerted a dominant negative effect by binding the HSPB1 wild-type decreasing the abundance of wild-type dimers. Interestingly, with canavanine-induced protein aggregation, fibroblasts heterozygous for the truncated p.Met169Cysfs*2 mutant displayed decreased cell viability [Ylikallio et al., 2015].

In this report, we show that the HSPB1 mutation p.Ala204Glyfs*6 leads to a loss of chaperone-like activity. Intriguingly, the first report of ALS-associated single nucleotide polymorphism (SNP) in HSPB1 suggested a similar feature. Dierick and collaborators previously reported the SNP rs545738637 T>C in the *HSPB1* promoter in a Belgian sALS patient. This variant is located within the heat-shock element (HSE), a consensus sequence for the specific binding of the heat-shock factor 1 (HSF1). The binding of HSF1 on HSE is essential for the upregulation of HSPB1 upon stress. The presence of the SNP rs545738637 T>C leads to the impairment of *HSPB1* stress-related increased transcription, thus resulting in a severely affected heat-shock response [Dierick et al., 2007].

Taking these data and our findings together, it becomes tempting to speculate about a correlation between different pathomechanisms of *HSPB1* mutations and the resulting neurodegenerative phenotype. The CMT-associated *HSPB1* mutations result in a hyperactive protein, enhancing the binding to their client proteins and leading to downstream effects detrimental in peripheral neurons. On the contrary, an impaired heat-shock response, either due to the lack of HSPB1 upregulation during stress conditions or resulting from a loss of chaperone-like activity, might contribute to the pathogenesis of ALS making the motor neurons more vulnerable to homeostasis perturbation. It is well documented that the aging-related accumulation of misfolded/oxidized proteins is a challenge to the proteostasis system, in particular in long-living cells like motor neurons. This is due to the fact that the age-related decline in the proteostasis machinery determines the inability to upregulate chaperones in response to conformational stress [Hartl et al., 2011]. In this scenario, the presence of *HSPB1* mutations would trigger the disease manifestation, perturbing central proteostasis, including folding and clearance mechanisms. Interestingly, the impairment of protein degradation pathways have already been linked to the pathogenesis of ALS through the identification of mutations in the ubiquitin-like protein *Ubiquilin2* (*UBQLN2*) [Deng et al., 2011] and the autophagy-related *Sequestosome 1* (*SQSTM1*) [Fecto et al., 2011] in familial and isolated cases.

ALS is a complex disease and the interactions among several genes influence its pathogenesis, providing a different effect on the phenotypic presentation [Leblond et al., 2014]. In regard to the complexity to decipher the genetic landscape of sALS, the investigation of the cellular implications of rare but pathogenic mutations can provide a novel approach to evaluate relevant molecular pathways in this sporadic disease. The investigation of the *HSPB1* gene in broader ALS/ALS-plus cohorts might therefore offer additional insights into deciphering the molecular basis of this motor neuron Disorder.

Acknowledgments

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