DNAJ proteins in neurodegeneration: essential and protective factors

Running title: DNAJ proteins and neurodegeneration

Christina Zarouchlioti*, David A. Parfitt*, Wenwen Li, Lauren M. Gittings, and Michael E. Cheetham†

UCL Institute of Ophthalmology, 11-43 Bath Street, London, EC1V 9EL UK

† To whom correspondence should be addressed:
Professor Mike Cheetham, UCL Institute of Ophthalmology, London, EC1V 9EL, UK
Tel: +44 2070686944; Fax +44 2076086892; Email: michael.cheetham@ucl.ac.uk

* These authors contributed equally to this work

Keywords: DNAJ, molecular chaperones, neurodegeneration

Abstract

Maintenance of protein homeostasis is vitally important in post-mitotic cells, particularly neurons. Neurodegenerative diseases such as polyglutamine expansion disorders, like Huntington's disease or spinocerebellar ataxia (SCA), Alzheimer's disease, fronto-temporal dementia (FTD), amyotrophic lateral sclerosis (ALS) and Parkinson's disease, are often characterized by the presence of inclusions of aggregated protein. Neurons contain complex protein networks dedicated to protein quality control and maintaining protein homeostasis, or proteostasis. Molecular chaperones are a class of proteins with prominent roles in maintaining proteostasis, which act to bind and shield hydrophobic regions of nascent or misfolded proteins while allowing correct folding, conformational changes and enabling quality control. There are many different families of molecular chaperones with multiple functions in proteostasis. The DNAJ family of molecular chaperones is the largest chaperone family and is defined by the J-domain, which regulates the function of HSP70 chaperones. DNAJ proteins can also have multiple other protein domains such as ubiquitin-interacting motifs or clathrin-binding domains leading to diverse and specific roles in the cell, including targeting client proteins for degradation via the proteasome, chaperone mediated autophagy and uncoating clathrin coated vesicles. DNAJ proteins can also contain ER-signal peptides or mitochondrial leader sequences, targeting them to specific organelles in the cell. In this review, we will

discuss the multiple roles of DNAJ proteins and in particular focus on the role of DNAJ proteins in protecting against neurodegenerative diseases caused by misfolded proteins. We will also discuss the role of DNAJ proteins as direct causes of inherited neurodegeneration via mutations in *DNAJ* family genes.

1. Introduction

Intracellular or extracellular proteinaceous inclusions in specific brain regions are a pathological hallmark of many neurodegenerative diseases [1]. These inclusions are generally composed of misfolded and aggregated forms of specific disease-associated proteins. For example, Alzheimer's disease (AD) is characterised by the accumulation of extracellular amyloid-β plaques and intracellular tangles of phosphorylated tau; Parkinson's disease (PD) is associated with intracellular deposits of α-synuclein known as Lewy bodies; and in Huntington's disease (HD) intracellular aggregates of polyglutamine expanded forms of the huntingtin protein are present. Protein aggregation in these neurodegenerative diseases can arise from genetic variations in the disease-related proteins (either as directly causative mutations or polymorphisms that shift the folding equilibrium of the disease-linked protein); genetic alterations that lead to elevated levels of the protein expression; or can be triggered by environmental stress and aging [2].

It is not always clear whether protein aggregation into inclusions is a cause or consequence of neurodegeneration; however, in inherited forms of neurodegeneration many of the causative mutations disrupt the folding of the disease protein leading to increased aggregation and inclusion formation. The pathological inclusions seen in all neurodegenerative disorders are thought to represent the end point of the protein aggregation process. Prior to the formation of large aggregates, mutated or misfolded proteins are believed to form small soluble oligomers, which some studies have demonstrated to be the more toxic species [3, 4]. It has been suggested, therefore, that the proteinaceous inclusions seen pathologically are not the primary cause of neurotoxicity, and their formation is a protective defence mechanism employed by the cell to sequester the potentially more toxic soluble oligomers [2]. Nevertheless, it is likely that these inclusions can also contribute to toxicity in neurons by physically obstructing axonal transport, sequestering other essential proteins and disrupting overall protein homeostasis of the cell. Neurons are a particularly vulnerable to this toxicity as they rely heavily on axonal transport between the cell body and synaptic terminals, and being a post-mitotic cell type, they do not have an ability to disperse protein aggregates via cell division, or be readily replaced [5].

Given their vulnerability to toxicity induced by aggregated oligomers and proteinaceous inclusions, neurons depend heavily on an intrinsic network of protein quality control mechanisms designed to maintain proteostasis; a state in which all proteins in the proteome are in a conformation, concentration and location that is required for correct functioning of the cell [6]. Cells have several mechanisms to regulate the biogenesis, folding, trafficking and degradation of proteins to ensure that proteostasis is maintained, and disruptions to these processes, or an imbalance in protein folding caused by mutations or stress, can lead to disease. Cells respond to stress through compartment related signalling pathways. In the cytoplasm and nucleus, the heat shock response (HSR) mediates a transcriptional response to stress through heat shock factors (e.g. HSF1), whereas, the endoplasmic reticulum (ER) has the unfolded protein response (UPR) to respond to stress [7, 8]. Intrinsic degradation mechanisms employed to maintain proteostasis include clearance systems such as autophagy and the ubiquitin-proteasome system (UPS), which involve the compartmentalisation, degradation and recycling of misfolded or unfolded proteins by lysosomes or proteasome, respectively [5, 9-11]. The HSR and UPR act to restore protein homeostasis by reducing protein translation and activating signalling pathways that increase production of protective factors, such as molecular chaperones [8].

Molecular chaperones are heterogeneous and functionally diverse families of proteins that are involved in many critical cellular processes, including protein folding, trafficking, quality control and degradation. A common classification of molecular chaperones (also known as heat shock proteins; HSPs) is according to their molecular weight. The major families are HSP90, HSP70, HSP40 (DNAJ), HSP60 and the small HSPs. In this review, the focus will be on the DNAJ family members and their relation to neuronal proteostasis and neurodegeneration [12].

2. DNAJ proteins

DNAJ proteins (also known as J proteins or HSP40 proteins) are a family of chaperones that regulate HSP70 chaperones through stimulating ATP hydrolysis. The defining feature of DNAJ proteins is the J-domain, an approximately 70 amino acid highly conserved region containing 4 α-helices (Figure 1). The linker region between helices 2 and 3 is especially well conserved and contains the histidine-proline-aspartic acid (HPD) motif that is absolutely required for stimulation of ATP hydrolysis in HSP70 [13]. There are approximately 50 different members of the DNAJ protein family in man, ranging in size from 10 to 520 kDa, suggesting that the HSP40

designation might not be an accurate description of this family of proteins [14]. The variety in size reflects the diversity in function of DNAJ proteins due to their varying domain structure [15].

DNAJ protein family members can be divided into three subtypes depending on their domain composition (class I, II or III, also called A, B or C; [16]) (Figure 1). Class I (DNAJA) DNAJ proteins are the most similar to the eponymous E. coli DnaJ protein and contain the canonical domain structure of an N-terminal J-domain followed by a glycine/phenylalanine (G/F)-rich region, a zinc-finger motif and C-terminal client-binding domain (CBD). Class II (DNAJB) DNAJ proteins contain an N-terminal J-domain and G/F-rich region. Class III (DNAJC) DNAJ proteins only have the J-domain with no other canonical domains, and the J-domain may be located anywhere in the structure of the protein. DNAJC proteins are the largest subtype of DNAJ proteins and have the greatest diversity in their size, structure and domain architecture, reflecting highly specialized functions. Among the wide variety of protein domains found in DNAJ proteins are ubiquitin-interacting motifs (UIMs), cysteine-rich regions, GTP-binding domains, tetratricopepetide repeats (TPRs) and clathrin-binding domains [17].

3. Mutations in DNAJ proteins as a cause of disease

Mutations in DNAJ proteins can cause disease, as part of a larger collection of genetically inherited disorders caused by mutations in molecular chaperones known as chaperonopathies [18]. Furthermore, the majority of chaperonopathies result in neurodegenerative-like phenotypes, emphasizing the important role of molecular chaperones in neuronal proteostasis, in particular motor neurons [19]. Currently mutations are known to occur in fourteen DNAJ proteins (Table 1, Figure 2), leading to diseases such as cerebellar ataxia, distal hereditary motor neuropathy, Charcot Marie Tooth disease, and Parkinson's disease [20]. However, mutations in some DNAJ proteins cause non-neurodegenerative disorders; for example mutations in *DNAJB13* cause primary ciliary dyskinesia [21], mutations in *DNAJC12* cause hyperphenylalaneimia [22] and mutations in *DNAJC21* cause bone marrow failure syndrome [23]. In this section, we will focus on the role of DNAJ mutations in contributing to neurodegeneration and the consequences for neuronal proteostasis.

(a) DNAJB2 (HSJ1)

DNAJB2 is an alternatively spliced neuronal protein forming two isoforms: a 36 kDa cytosolic/nuclear form (DNAJB2a; HSJ1a) and a larger 42 kDa isoprenylated membrane associated form (DNAJB2b; HSJ1b) [24]. As a type II DNAJ protein it contains an N-terminal J-domain and G/F-rich region, but also a CBD (that is not

conserved with DNAJ) and two ubiquitin-interacting motifs (UIMs), which can bind ubiquitylated client proteins and target them to the proteasome for degradation [25]. There are several biallelic mutations known in *DNAJB2* that are associated with a range of neurodegenerative diseases. Charcot Marie Tooth (CMT) disease results in progressive degeneration of spinal cord motor neurons, leading to weakness and muscle atrophy in the lower limbs [26]. Patients also show distal sensory loss. A homozygous missense mutation c.14A>G in *DNAJB2* resulting in a substitution of tyrosine for cysteine at residue five (Y5C) in the DNAJB2 J-domain causes CMT type 2 [27, 28]. There are also reports of splicing mutations in *DNAJB2* resulting in distal hereditary motor neuropathies (dHMN), which are a genetically and clinically heterogeneous group of disorders similar to CMT, but without the sensory abnormalities [29, 30]. A homozygous splice site mutation (c.352+1G>A) was identified in *DNAJB2* leading to either partial or total retention of intron 5, resulting in reduced DNAJB2 protein expression [31]. There have been additional reports of patients with this mutation recently [32]. This mutation has been suggested to be a potential founder mutation, because in another study of CMT/dHMN the five affected individuals with this mutation shared a common haplotype [33]. Similarly, Gess et al reported a dHMN patient with a homozygous c.229+1G>A *DNAJB2* splice site mutation, leading to the retention of intron 4 and subsequent loss of DNAJB2 protein expression [28]. A recent study identified a large-scale deletion incorporating the first four exons of *DNAJB2* (including the entire J-domain) as causing spinal muscular atrophy (SMA) and atypical juvenile parkinsonism (AJP) [34]. A recent exome analysis of peripheral neuropathy patients identified two new mutations in DNAJB2; a frameshift truncation (F103fsX) and a splice site mutation (c.619-1G>A; [35]).

(b) DNAJB5 (HSC40)

DNAJB5 was originally identified containing similarity to DNAJB1 [36] and has since been shown to interact with HSP70 [37]. A whole-exome sequencing analysis of CMT-like patients identified a mutation in the J-domain of DNAJB5 (P15S) as a novel cause of neuropathy [35]. Morpholino-mediated knockdown of DNAJB5 in zebrafish revealed abnormalities in peripheral nerve axon structure, but no effect on muscle architecture [35]

(c) DNAJB6 (MRJ)

DNAJB6 is a ubiquitous protein with high expression levels in the brain, and detectable protein in muscle [38]. Alternative splicing of the DNAJB6 gene produces two isoforms: a 36 kDa nuclear isoform and a 26 kDa cell stress-responsive cytosolic form [39]. Mutations in *DNAJB6* cause limb-girdle muscular dystrophy type 1 (LGMD1). LGMD1 is an autosomal dominant disease characterized by progressive

distal and occasionally proximal muscle atrophy caused by myofibrillar myopathy. There is also a report of a DNAJB6 patient with frontotemporal dementia alongside LGMD1 [40]. There are currently twelve mutations known in *DNAJB6* (Table 1); interestingly, all of the mutations are found in exon 5, which codes for the G/F-rich region of the protein. Ruggieri and colleagues have suggested that there might be a genotype-phenotype correlation between both the severity of the disease and the location (proximal-distal) and the mutated residue involved, with C-terminal mutations leading to a distal phenotype [41]. Patients with *DNAJB6* mutations have myofibrillar aggregates containing ubiquitin, TDP-43 and p62, suggesting defective protein clearance [42, 43], which are also observed in *Dnajb6* F93L transgenic mice [44]. *Drosophila* mutants recapitulating patient mutations result in loss of DNAJB6-dependent anti-aggregation activity [45].

(d) DNAJC3 (p58)

DNAJC3 is a 58 kDa DNAJ protein that is targeted to the cytoplasmic face of the ER [46, 47]. DNAJC3 can also bind and inhibit the UPR sensor PERK in the ER, suggesting a role in regulating the UPR [48, 49]. DNAJC3 can recruit cytosolic HSP70 to the face of the ER and work with Sec61 as part of the translocation machinery [50]. Knockdown of DNAJC3 results in accumulation of misfolded protein in the ER and activation of the UPR [51] and *Dnajc3* knockout mice have decreased ability to cope with ER stress [50, 52]. Mutations in DNAJC3 cause multisystemic neurodegeneration, including early onset cerebellar ataxia and peripheral neuropathy, alongside diabetes mellitus [53]. Interestingly, *Dnajc3* knockout mice also show a diabetes phenotype [52] and recent work has also shown that the ubiquitin ligase CHIP is involved in the turnover of the insulin receptor, suggesting a link between proteostasis network control and insulin regulation [54].

(e) DNAJC5 (CSPα)

DNAJC5 is a secretory vesicle protein found in both neuronal and non-neuronal tissues; however, the main α-isoform is only expressed in the brain [55]. DNAJC5 is characterized by a cysteine-rich region and is targeted to post-Golgi membranes via palmitoylation [56]. DNAJC5 has a role in binding and folding many proteins required at the synapse, such as SNAP-25, syntaxins and synaptotagmins [57-59], where it acts as a co-chaperone with the constitutive HSP70, HSC70 (HSPA8) [60]. DNAJC5, therefore, most likely plays a key role at the synapse as a chaperone [61, 62]. Mutations in *DNAJC5* cause autosomal dominant adult onset neuronal ceroid lipofusinosis (ANCL), an accumulation of autofluorescent lysosomal waste (known as lipofuscin) that causes a progressive neurodegenerative disorder characterized by ataxia, seizures and dementia [63]. ANCL is a rare disease and to date only two

distinct mutations in *DNAJC5* (deletion of leucine 116 and missense change L115R) have been identified in a handful of families [64-67]. The location of the mutations in the cysteine-rich region suggests a defect in the membrane trafficking of patient DNAJC5 and subsequent protein aggregation [68, 69]. DNAJC5 interacts with another ANCL disease-causing protein, palmitoyl-protein thioesterase 1 (PPT1). PPT1 with decreased activity is accumulated in DNAJC5 patient brains, suggesting a link between ANCL and palmitoylation of synaptic proteins [70]. *Dnajc5* KO mice have deficient neuromuscular function and sensorimotor impairment. Indeed, these mice have specific degeneration of the neuromuscular junctions, implying that KO of *Dnajc5* leads to synapse dysfunction [55]. Interestingly, DNAJC5 mutations in *C. elegans* leading to sensory neuron dysfunction could be rescued by treatment with resveratrol [71].

(f) DNAJC6 (auxilin)

Another well-characterised vesicle associated protein is DNAJC6, which has a role in uncoating clathrin-coated vesicles [72]. DNAJC6 binds clathrin via its C-terminal clathrin binding-domain [73, 74]. The clathrin-coating and uncoating cycle is well characterised; clathrin triskelions form coated pits at the pre-synaptic membrane around the intended cargo. Before fusing with endosome, the vesicles need to be uncoated by HSC70, following recruitment and activation by DNAJC6 [75, 76]. In neurons, this process is vital for synaptic vesicle recycling. Mutations in *DNAJC6* were first associated with autosomal recessive juvenile parkinsonism (ARJP) [77, 78]. Symptoms of ARJP include typical PD features, but also include mental retardation and seizures. ARJP typically manifests in the first decade and rapidly leaves patients wheelchair-bound. There is also a report of a 80 kb large-scale deletion including *DNAJC6* that results in ARJP [79]. A recent study also identified variants in *DNAJC6* that are associated with early-onset PD, which has a later onset than ARJP [80]. The authors suggest that this may be due to residual DNAJC6 activity compared to the ARJP mutations, which likely cause complete loss of function, and therefore represents a genotype-phenotype correlation of *DNAJC6* mutations. Interestingly, a mutation in a highly conserved residue (R927G) in the J-domain was found that potentially disrupts the HSC70 interaction.

(g) DNAJC11

DNAJC11 was originally described as a 63 kDa protein containing an N-terminal J-domain that is often deleted in neuroblastoma [81, 82]. DNAJC11 was later identified as a mitochondrial protein [83], specifically as a member of the mitochondrial complex I, involved in the electron transport chain, although siRNA-mediated knockdown had no effect on the assembly of the complex [84]. Using random N-

ethyl-N-nitrosurea (ENU) mutagenesis, Ioakeimidis et al created a spastic mouse model with a deep intronic mutation in *Dnajc11*, resulting in the addition of a 109 bp cryptic exon and a frameshift truncation and reduction of Dnajc11 protein [85]. These mice had abnormal locomotion and progressive muscle wasting and spasticity resulting in death at five weeks of age. They also had highly vacuolated motor neurons in the lumbar spinal cord, generated from either abnormal mitochondrial cristae or ER, as mitochondria in these motor neurons were severely disrupted [85].

(h) DNAJC13 (RME-8)

DNAJC13 is an endocytic protein that has been shown to localize to early and recycling endosomes [86]. The J-domain of DNAJC13 is located in the middle of the protein; with a membrane-binding region at the N-terminus and four potential clathrin-binding motifs [86]. DNAJC13 interacts with the retromer complex [87] and thus may have a role in recruiting HSC70 to vesicle formation sites. An inherited variant (N855S) in *DNAJC13* was originally thought to cause autosomal-dominant PD [88]; however, two affected family members did not have this variant and subsequent whole-exome sequencing identified two causative changes in another endosomal/synaptic protein TMEM230, questioning the importance of this variant for PD [89]. However, sequence analysis of exon 24 of *DNAJC13* in a Caucasian population study has suggested that N855S could be a rare variant associated with PD [90]. Further analysis revealed that other DNAJC13 variants (E1740Q, R1615H, L2120W) might be associated with increased risk of PD [91, 92].

(i) DNAJC19 (TIM14)

DNAJC19 is one of several mitochondrial DNAJ proteins, found at the inner mitochondrial membrane. It recruits and activates mitochondrial HSP70 (HSPA9) to function as part of the mitochondrial import machinery [93]. Mutations in DNAJC19 cause autosomal recessive dilated cardiomyopathy and cerebellar ataxia (DMCA). A splice site change that leads to the loss of exon 4 and subsequent truncation of the protein was the first mutation identified [94]. Recently, single nucleotide deletions and splice deletions have also been identified with associated disease features [95-97].

(j) DNAJC29 (sacsin)

The largest known DNAJ protein is DNAJC29 (520 kDa), which contains a C-terminal J-domain, an N-terminal ubiquitin-like (UbL) domain, three sacsin repeat regions (SRRs), which show homology to the ATP-binding domain of HSP90, and a C-terminal higher eukaryote and prokaryote (HEPN) domain [98, 99]. DNAJC29 is a neuronal protein that is localized to the cytoplasmic face of the mitochondria; knockdown of DNAC29 results in disruption of the mitochondrial network [100]. Mutations in *DNAJC29* cause autosomal recessive spastic ataxia of Charlevoix-

Saguenay (ARSACS), an early onset disorder characterized by cerebellar ataxia and peripheral neuropathy, with prominent Purinkje cell death in the cerebellum [101]. There is a large founder effect in the patient population; the vast majority of patients are from the Quebec region in Canada and have the R2502X mutation [102], although more than 150 other patient mutations are now known worldwide, including large-scale deletions [103, 104]. Mutations in DNAJC29 are the second most common cause of autosomal recessive ataxia after mutations in frataxin, which causes Freidrich's ataxia. The J-domain of DNAJC29 has been shown to functional via a bacterial complementation assay, and interestingly there are two patient missense mutations located in the J-domain (R4331Q and E4343K) [105, 106]. *Dnajc29* knockout mice have ataxic symptoms with peripheral neuropathy and progressive Purkinje cell loss, recapitulating the human disorder [107]. Furthermore, *Dnajc29* null mice motor neurons have elongated mitochondria and accumulations of neurofilaments [107]. DNAJC29 interacts with the mitochondrial fission protein DRP1 [100] and recent work using patient fibroblasts has shown that there is a reduction of DPR foci at the mitochondria and mitochondrial health and function in ARSACS are decreased, suggesting impairment in the ability of the mitochondrial network in affected neurons [108].

4. Manipulation of DNAJ proteins in models of neurodegeneration

The late onset of many neurodegenerative diseases has been suggested to correlate with a reduced efficiency of the protein quality control machinery as a result of aging. Correspondingly, the manipulation of molecular chaperones is a promising therapeutic approach for many neurodegenerative diseases [12]. Recently, several studies have focused on increasing the expression of chaperones in different neurodegeneration models and the data support the potential of chaperone manipulation, and in particular DNAJ proteins, in the battle against neurodegenerative diseases. In this section, the focus will be on targeting different disease-related proteins in neurodegeneration with members of the DNAJ chaperone family.

(a) Polyglutamine (polyQ) expansion disorders

The polyglutamine (polyQ) disorders are a group of neurodegenerative diseases caused by a trinucleotide CAG repeat expansion that confers a toxic gain-of-function, with a direct relationship between the length of the polyQ expansion and the propensity to aggregate. PolyQ expansions have been identified in Huntington's disease (HD; huntingtin, htt), spinal and bulbar muscular dystrophy (SMBA; androgen

receptor, AR) and spinocerebellar ataxias (SCA1, SCA2, SCA3, SCA7, ataxin; SCA6, CACNA1A; SCA17, TBP). Ubiquitylated inclusions of aggregated protein are characteristic of these diseases [109].

Manipulation of polyQ protein aggregates by molecular chaperones was first reported by Cummings *et al.* in 1998. Overexpression of DNAJA1 in cells reduced aggregation of polyQ expanded ataxin-1 (SCA1) [110]. In cells overexpressing polyQ-expanded ataxin-1, knockdown of DNAJC29 has been shown to enhance ataxin-1-mediated toxicity indicating a protective role against poly-Q expanded ataxin-1 toxicity [98]. DNAJB2a has been shown to have a dual role on ataxin-3 (Atx3; SCA3) depending on the chaperone's domains. In cells, DNAJB2a can either reduce the protein levels of Atx3 by promoting proteosomal degradation through J-domain, or diminish this process by preserving ubiquitylated Atx3 via the UIM domain [111]. Interestingly, DNAJA1 has also been reported to increase polyQ aggregation depending on the cell line used, an effect attributed to the J-domain that is responsible for the recruitment of endogenous HSP70 [112]. Since DNAJ chaperones are co-chaperones of HSP70, differences in levels of endogenous expression of HSP70 and DNAJ proteins between cell lines could explain this effect, as it could depend on the cellular chaperone balance. Similarly, overexpression of DNAJB1 in Neuro2a cells suppressed htt inclusion formation, while simultaneous overexpression of HSP70 improved folding efficiency and cellular proliferation and reduced cytotoxicity [113, 114]. DNAJB1 has been also shown to increase solubility of polyQ expanded androgen receptor (AR) and also enhance proteasome-mediated degradation in cells, an effect again amplified in the presence of HSP70 [115, 116].

A screen of several different DNAJA and DNAJB proteins revealed that a subfamily of DNAJB proteins were the most efficient at reducing polyQ aggregation [37, 117]. This subfamily includes DNAJB2a, DNAJB6b and DNAJB8, which are closely related (Figure 1C), but the effect is also dependent on their sub-cellular localisation. *In vitro* studies with purified proteins have shown that DNAJB6b can supress the formation of amyloid-like fibrils of polyQ peptides [118]. Moreover, DNAJB6b and DNAJB8 were shown to suppress polyQ aggregation and related toxicity in cells and transgenic *Xenopus laevis* models [117]. Genome-wide RNA interference screen on transgenic *C.elegans* expressing polyQ proteins identified DNAJ as a suppressor of polyQ aggregation [119]. Interestingly, DNAJB6b and DNAJB8 are effective in suppressing the aggregation not only of polyQ-expanded htt, but also of other disease-related poly-Q expanded proteins, such as Atx3 and the androgen receptor (SBMA) [117]. DNAJB6 and DNAJB8 were suggested to act on earlier stages of aggregation in cells despite their irreversible recruitment on larger

aggregates in an unsuccessful attempt to prevent aggregation [120]. Furthermore, the cytoplasmic/nuclear DNAJB2 isoform, DNAJB2a, is recruited to polyQ inclusions and can reduce the polyQ aggregation and inclusion incidence in a cellular overexpression model in a J-domain and UIM independent manner by promoting degradation via the proteasome [25, 121]. DNAJB2b has been also shown to inhibit neuronal death caused from mutant htt *in vitro* and also improve neuronal dysfunction in a *C. elegans* model of HD independent of any effect on polyQ aggregation [121]. *In vitro* studies have suggested that HSP70 and DNAJB1 can act on early stages of polyQ aggregation by halting or suppressing the formation of detergent-insoluble amyloid-like fibrils of polyQ [122].

In vivo investigation in *Drosophila* models for HD identified dHDJ1, the homologue of DNAJB1, as a suppressor of polyQ-driven toxicity [123]. A separate study showed that the DNAJB1-induced reduction of eye degeneration in transgenic polyQ *Drosophila* was enhanced by *Drosophila* HSC70cb and its human homolog APG-1, while DNAJB1 also had an effect in the absence of HSP70 [124]. Moreover, dMRJ, the *Drosophila* ortholog of the human DNAJB6, was recruited in the polyQ inclusions and was shown to suppress polyQ-mediated toxicity in flies [125]. In the same model, early expression of dHDJ1 dramatically promoted cytoplasmic aggregation of polyQ, while both DNAJ chaperones increased the level of detergent-soluble polyQ, illustrating the similarities and diversity of DNAJ chaperones [125]. Expression of dHDJ1 on mutant Atx3 expressing flies restored eye structure, an effect attributed to both J- and C- terminal domains. Interestingly, dHDJ1 effect on toxicity is enhanced in the presence of HSP70 and abolished in the presence of mutant HSP70. Both dHDJ1 and HSP70 overexpression altered the solubility of polyQ; however, expression of dHDJ2, that has the same J-domain but different C-terminal domains, resulted in weak suppression of eye degeneration in the flies suggesting a role of the C-terminal domain [126]. In *Drosophila* models of SCA6 that express a CAG expansion in exon 47 of *CACNA1A* (a1ACT), DNAJ-1 was shown to suppress a1ACT-induced toxicity in the eye, while DNAJ-1 knockdown dramatically accelerated eye degeneration [127]. Interestingly, normal Atx3 has been shown to alleviate toxicity of several polyQ-expanded disease proteins including itself and mutated htt. Atx3 interacts with Rab23 which leads to increased DNAJ levels which in turn leads to reduced eye degeneration in flies [128].

Despite these promising effects in other models, few direct chaperone overexpression experiments have successfully translated to the mammalian nervous system. DNAJB2a was effective in reducing polyQ inclusion formation in a rat brain model of SBMA using viral delivery, by increasing ubiquitylation and targeting to the

UPS [129]. Moreover, two members of the DNAJ family have been shown to be effective on polyQ aggregation in the R6/2 transgenic mouse model of HD [130, 131]. Transgenic overexpression of human DNAJB2a led to a reduction in polyQ aggregation and inclusion size in the cortex and striatum of R6/2 mice at 15 weeks of age and led an increase in htt solubility; however, the improvement in the neurological performance was relatively modest and there was no increase in lifespan [130]. Immunopurification of htt from mouse brain and combinations of purified polyQ protein with cell or mouse brain extracts suggested that the maximal DNAJB2 effect required functional J and UIM domains, and that the effect was mainly being mediated on preformed aggregates, preventing further seeding of aggregation [130]. A recent study on transgenic R6/2 mice overexpressing human DNAJB6 also showed a reduction in inclusion formation in the brain accompanied by improved neurological performance and increased life-span [131]. *In vitro* studies suggest this was through an effect on primary nucleation of polyQ aggregation [131] The differences in the magnitude of the neurological effect between the DNAJB2a and DNAJB6 R6/2 mice could be attributed either to differences in the mechanism of action of the two chaperones, differences in the level of the transgene expression (as different promoters were used), or differences in chaperone regulation. For example, recently DNAJB2a has been shown to be a target of the ubiquitously expressed kinase CK2. CK2 phosphorylated DNAJB2 in the second UIM and reduced its ability to bind ubiquitylated clients [132]. Therefore, it is possible that the maximal activity of DNAJB2a was repressed by CK2 and that inhibition of CK2 could amplify the effect of DNAJB2.

(b) α-synuclein and Parkin in Parkinson's disease

Parkinson's disease (PD) is the second most common neurodegenerative disorder. Although most cases of Parkinson's disease are sporadic, α-synuclein is the main component of Lewy bodies, which are ubiquitin-positive cytoplasmic inclusions formed in patients with PD, Lewy body dementia and other disorders [133]. Furthermore, mutations in *SNCA*, which encodes α-synuclein, have been associated with PD and mutations in *PARK2*, which encodes Parkin, lead to autosomal recessive juvenile form of the disease [134].

DNAJB1 has been shown to slow down the assembly of α-synuclein fibrils and increase the binding of HSC70 to fibrillar α-synuclein *in vitro* [135]. In addition, DNAJA1 was reported to bind α-synuclein fibrils and increase binding of HSC70 to preformed fibrils *in vitro*; however, DNAJA1 alone had no effect on the assembly of α-synuclein fibrils [135]. Both DNAJA1 and DNAJB1 have been shown to co-localise

with α-synuclein inclusions in cells [136]. Post-mortem PD brain tissue showed immunoreactivity for both DNAJB1 and DNAJB6 in Lewy bodies, while DNAJB1 was also present in Lewy neurites and DNAJB6 was upregulated in astrocytes indicating a potential role in the disease [137, 138]. Moreover, co-expression of α-synuclein and either DNAJA1 or DNAJB1 dramatically decreased α-synuclein aggregates in cells [136]. Finally, DNAJB1 combined with HSP70 and HSP110 can recover amorphous α-synuclein aggregates, while they also enhance the effect of non-mammalian Hsp104 to remodel α-synuclein amyloids *in vitro* [139]. *In vivo* overexpression of the human homolog DNAJC10 in *C. elegans* decreased α-synuclein aggregates and toxicity [140].

Mutations in *PARK2* cause ARJPD, *PARK2* encodes Parkin, a ubiquitin E3 protein ligase containing a N-terminal ubiquitin-like domain and two C-terminal RING finger domains that plays an important role in mitochondria dynamics and function [141]. DNAJB2a expression was effective in reducing misfolding and aggregation of RING1 domain mutant Parkin in cells. Furthermore, in the presence of DNAJB2a, mutant Parkin was relocalised to mitochondria and its ability to promote mitophagy of damaged mitochondria was significantly restored [142]. In contrast to polyQ, most cytosolic DNAJ proteins tested could reduce mutant Parkin RING1 domain mutant (C289G) aggregation, and for DNAJB6 and DNAJB8 this was less reliant of their S/T region and more dependent on HSP70 [143]. This illustrates that chaperone manipulation can be versatile and unique to individual protein clients.

(c) Tau and amyloid-β

Extracellular amyloid plaques composed of amyloid-β (Aβ) peptides and intraneuronal tau neurofibrillary tangles form the characteristic pathophysiological profile of Alzheimer's disease (AD) [144]. Although accumulation of Aβ fibrils occurs extracellularly on senile plaques, intraneuronal generation of Aβ has been correlated to synapse damage and enhanced intracellular accumulation in AD-transgenic mice [145].

Interestingly, DNAJB1 has been shown to enhance the effect of HSP70 *in vitro* in reducing Aβ aggregation through targeting smaller species such as oligomers [146]. Moreover, DNAJB6, and specifically the DNAJB6b isoform, which is localised in both the nucleus and the cytosol, has been shown to be a potent suppressor of Aβ42 aggregation *in vitro* preventing the formation of amyloid fibrils by interacting with the early formed aggregates during nucleation [147]. In a cellular model of AD overexpressing GFP-tagged Aβ42, DNAJB6 was shown to reduce intracellular Aβ aggregation and required interaction with HSP70. In *C. elegans* models of Aβ,

overexpression of DNAJ27 (ortholog of mammalian DNAJC10) had a protective role against Aβ-induced toxicity; however, overexpression of human DNAJC10 in Aβ worms had no effect [140].

DNAJA1 has been shown to act as a regulator of tau fate depending on HSP70 levels. More specifically, in the absence of HSP70, DNAJA1 enhanced ubiquitin-mediated proteolysis of mutant tau, while in the presence of HSP70, DNAJA1 stabilised tau and halts degradation [148]. Considering that it is still not clear whether aggregation of misfolded proteins is a protective or pathogenic mechanism for neurons, this dual potential of DNAJA1 could be of value in targeting AD pathogenesis. DNAJB1 had a dose-dependent effect on tau aggregation *in vitro* [149]. Finally, Brehme *et al* have shown that knockdown of DNAJA1 and DNAJA4 or the *C. elegans* homologues can increase the aggregation and toxicity of Aβ42 [150].

(d) SOD1 and TDP-43

The misfolding and aggregation of TAR DNA-binding protein-43 kDa (TDP-43) and superoxide dismutase 1 (SOD1) are associated with amyotrophic lateral sclerosis (ALS), which presents with degeneration of the upper and lower motor neurons. In healthy individuals, TDP-43 appears predominately in the nucleus, while in disease TDP-43 forms ubiquitin positive nuclear and cytoplasmic inclusions with abnormal phosphorylation. In familial ALS, *SOD1* mutations lead to the formation of ubiquitin positive SOD1 inclusions in ALS patient spinal cord and in mouse models [151].

Both DNAJB2 isoforms have been shown to significantly reduce mutant SOD aggregation in an overexpression cell model [31, 152]. *In vivo* investigation of DNAJB2a overexpression in double transgenic $SOD1^{G93A}$ mice has shown that DNAJB2a can improve muscle function in late stages of the disease by improving the survival of motor neurons and muscle weight [152].

DNAJB1 co-immunopurified with mutant SOD1, but not with wild type or endogenous from cell extracts [153]. In the presence of Hsp70, DNAJB1 can reduce the formation of cytoplasmic aggregates of SOD1 and improve neurite outgrowth in a neuronal cell model (Neuro2a) [154]. Finally, Chen *et al.* showed that HSF-1 overexpression could reduce TDP-43 aggregation in HEK293 cells. A screen of several DNAJ chaperones revealed that overexpression of DNAJB2a was the most efficient at suppressing TDP-43 aggregation at similar levels to HSF-1 activation. It was suggested that DNAJB2a binds TDP-43 aggregates and delivers them to HSP70 for refolding via its J-domain and not for degradation [155].

5. Conclusions

The essential role of molecular chaperones in maintaining neuronal proteostasis is highlighted by the several disease-causing mutations in members of the DNAJ family. Moreover, several DNAJ proteins have been shown to be beneficial for restoring neuronal proteostasis and reducing neurotoxicity associated with a wide range of neurodegeneration proteins both *in vitro* and *in vivo*. The great diversity among DNAJ proteins might enable individual DNAJ proteins to be tailored to distinct aggregation-prone proteins. Conversely, some members of the DNAJ family, such as DNAJB2 and DNAJB6, appear to have the ability to affect a wide range of neurodegeneration related protein clients for potential therapeutic benefit. Enhanced understanding of the DNAJ family function and regulation in neurons is likely to lead to better application of these potentially critical architects of neuronal proteostasis.

6. Acknowledgments

Research on proteostasis in the Cheetham lab is supported by the MRC, Wellcome Trust and MNDA. LMG is a Leonard Wolfson Experimental Neurology PhD student and WL was a CSC PhD student. The authors wish to thank Zheng Shi of Chengdu University, China for help with creating the DNAJ phylograms.

7. Table 1. Mutations in DNAJ proteins cause a range of diseases.

DNAJ gene (AKA)	Mutation/Result	Disease	Inheritance	References
DNAJB1 (HDJ1/HSP40)	400kb deletion on chromosome 19 resulting in N-terminal DNAJB1 chimeric in-frame fusion with PKA catalytic domain	Fibrolamellar heptaocellular carcinoma	somatic	[154]
DNAJB2 (HSJ1)	c.352+1G>A resulting in intron 5 retention c.229+1G>A resulting in intron 4 retention	distal hereditary motor neuropathy	recessive	[31] [28] [34] [33] [35]
	c.14A>G, p.Y5C (J-domain mutation) c.619-1G>A resulting in splice site deletion c.309delC, p.F103fsX	Charcot Marie Tooth disease type 2		
	3.8kb deletion resulting in J-domain deletion	Spinal muscular atrophy/juvenile Parkinsonism		
DNAJB5 (HSC40)	c.43C>T, p.P15S (J-domain mutation)	hereditary myoclonus and progressive distal muscular atrophy	recessive	[35]
DNAJB6 (MRJ)	c.265T>A, p.F89I c.271T>A, p.F91I c.271T>C, p.F91L c.273C>G, p.F93I	Limb-girdle muscular dystrophy	dominant	[155] [156] [43] [157] [158] [159] [160] [161] [162]

	c.277T>A, c.277T>C, c.279C>A, c.279C>G, p.F93L c.287C>G, p.P96R c.287TC>T, p.P96L c.298T>G, p.F100V c.346+5G>A			
DNAJB13	c.833T>G, p.M278R c.68+1G>C, p.Y24X	Primary ciliary dyskinesia type 34	recessive	[21]
DNAJC3 (p58)	c.508C>T, p.R194X 72kb deletion resulting in loss of exons 6-12	combined cerebellar and peripheral ataxia with hearing loss and diabetes mellitus	recessive	[53]
DNAJC5 (CSPα)	c.346_348delCTC, p.L116Δ c.344T>G, p.L115R	adult-onset neuronal ceroid lipofuscinosis	dominant	[64] [63]
DNAJC6 (auxilin)	c.801-2A>G c.2371C>T, p.G791X	autosomal recessive juvenile Parkinsonism	recessive	[76] [77] [79] [163] [78]
	c.397A>T, p.M133L c.626T>C, p.L209P c.1468+83del c.1855C>T, p.R619C c.2038+3A>G resulting in loss of splice donor site c.2200C>T, p.G734X c.2365C>T, p.G789X	early onset Parkinsons disease		

	c.2223A>T, p.T741X c.2517del, p.F389LfsX22 c.2779A>G, p.R927G (J-domain mutation)			
	80kb deletion of exons 5-19	early onset obesity, mental retardation and epilepsy		
Dnajc11	c.1524+56T>A (mice only) resulting in cryptic splicing, p.K508fsX43	spasticity, MN pathology	recessive	[84]
DNAJC12 (JDP1)	c.298-968_503-2603del resulting in exon 4 deletion c.215G>C, p.R72P c.158-2A>T resulting in intron 3 splice site mutation	hyperphenylalaninemia, mild, non-BH4 deficient	recessive	[22]
DNAJC13 (RME-8)	c.2564A>G, p.N855R	autosomal dominant Parkinsons disease	dominant	[87]
DNAJC17	c.681G>A (r.601_681del), p.Y201_A227del	retinitis pigmentosa and hypogammaglobulinemia	recessive	[164]
DNAJC19 (TIM14)	IVS3-1G>C resulting in skip exon 4 and frameshift truncation c.300delA, p.A100fsX11 c.63delC, p.Y21X c.280+1_280+5delGTAAG resulting in splice site deletion	dilated cardiomyopathy and ataxia	recessive	[93] [165] [94] [95] [96]
DNAJC21	c.517C>T, p.R173X	Bone marrow failure	recessive	[23]

	c.983+1G>T, p.G299AfsX2 c.94C>G, p.P32A c.793G>T, p.Q265X	syndrome type 3		
DNAJC29 (sacsin)	c.7504C>T, p.R2502X c.8844delT, p.P2948fsX3 c.12992G>A, p.R4331Q (J-domain mutation) c.12991C>T, p.R4331W (J-domain mutation) c.13027G>A, p.E4343K (J-domain mutation) more than 150 other mutations	autosomal recessive spastic ataxia of Charlevoix-Saguenay	recessive	[101] [104] [105] [166] [167] [168]

8. Figures

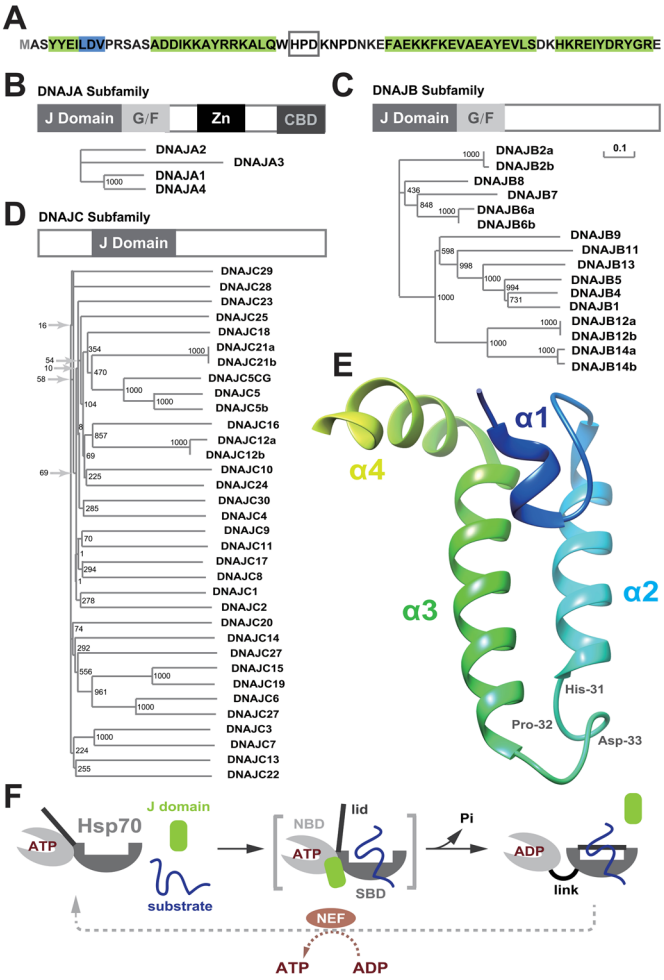

Figure 1. *The J domain and DNAJ subfamilies*. (**A**) The amino acid sequence of the DNAJB2 J domain with α-helixes (yellow), β-sheet (blue) and HPD motif (boxed) highlighted. (**B**-**D**) Phylograms of DNAJ subfamilies and schematic illustrations of their conserved domains. Protein sequence alignments were performed using a Blosum scoring matrix in ClustalX. Bootstrap value is presented at right corner in Section C. Numbers represent the degree of homology (0-1000). (**E**) The tertiary structure of J domain of DNAJB2 (PDB 2LGW) from N-terminus (dark blue) to C-terminus (yellow) is shown with the 4 α-helixes and HPD motif highlighted. (**F**) Illustration of how the J-domain (green) can facilitate substrate (dark blue) loading onto Hsp70 (grey). When the ATP is bound, the C-terminal substrate-binding domain (SBD) is docked onto the N-terminal nucleotide-binding domain (NBD). DNAJ proteins simulate Hsp70 ATPase hydrolysis, as well as recruiting substrates. When the ADP is bound, the lid closes and stabilizes the cleft-substrate binding. Nucleotide exchange factors (NEF) (dark red) complete the cycle by stimulating the exchange of ADP for ATP and substrate release.

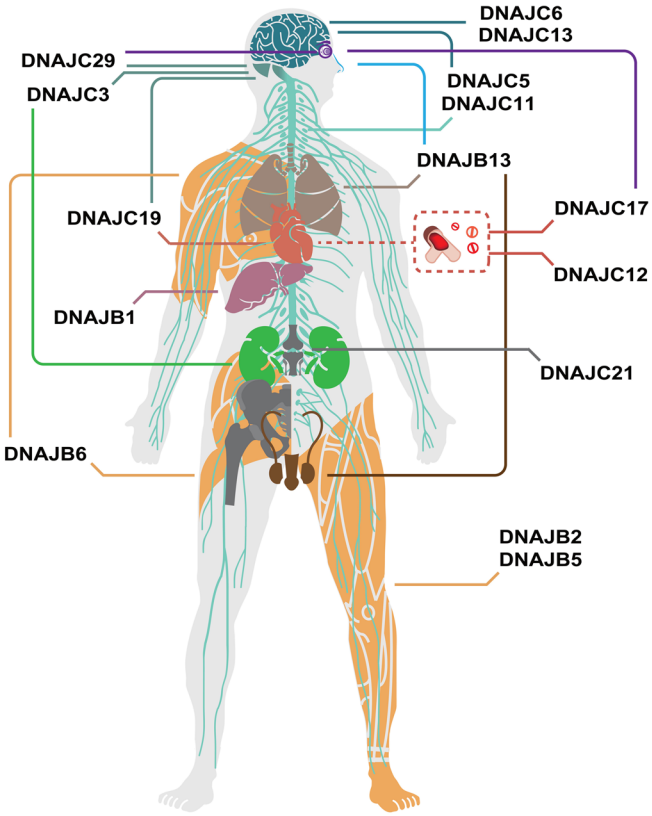

Figure 2. *Pathogenic consequences of DNAJ family mutations***.** Schematic illustration of a human body demonstrates the tissues and organs affected by DNAJ mutations, including nerve system composed of brain (dark cyan), cerebellum (cyan), spinal cord and peripheral nervous system (light cyan), eyes (purple), nose (blue), lungs (beige), heart, blood vessels (red), liver (dark pink), kidneys (green), muscles (orange), reproductive system (brown) and bones (grey).

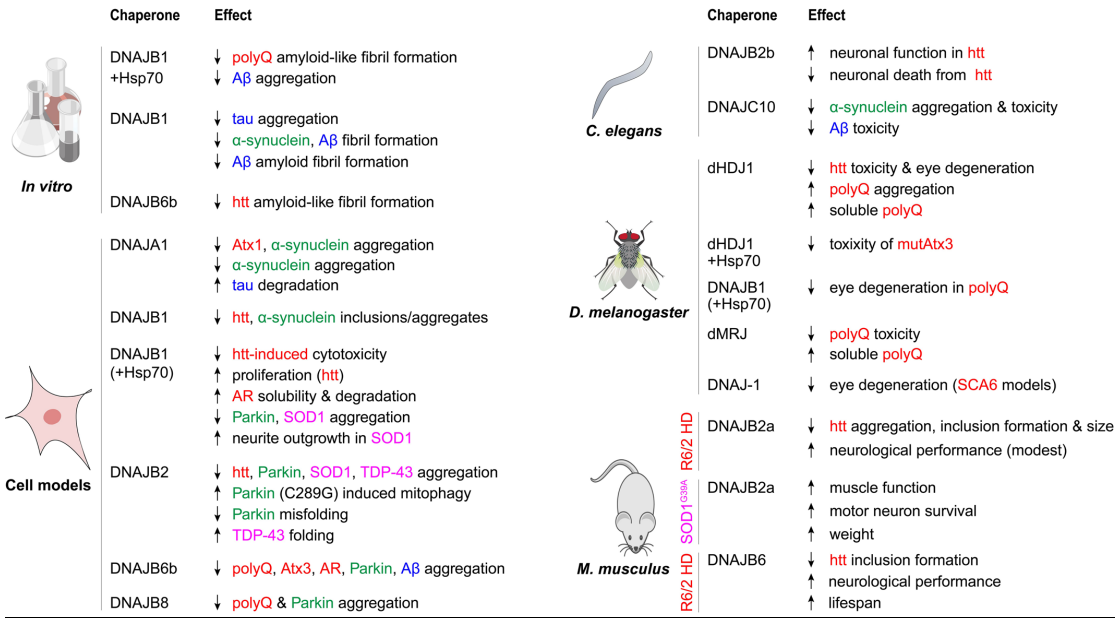

Figure 3. *DNAJ proteins modulate neurodegeneration in model systems*. Polyglutamine (polyQ) expansion models of huntingtin (htt), Ataxin-1 (Atx1), ataxin-3 (Atx3) androgen receptor (AR) and spinocerebellar ataxia type 6 (SCA6) shown in red. α-synuclein and parkin implicated in Parkinson's disease and amyloid-β (Aβ) and tau present in Alzheimer's disease in green and blue, respectively. TAR DNA-binding protein-43 kDa (TDP-43) and superoxide dismutase 1 (SOD1) present in amyotrophic lateral sclerosis in magenta.

9. References

[1] Soto, C. 2003 Unfolding the role of protein misfolding in neurodegenerative diseases. *Nat Rev Neurosci* **4**, 49-60. (DOI:10.1038/nrn1007).

[2] Ross, C. A. & Poirier, M. A. 2005 Opinion: What is the role of protein aggregation in neurodegeneration? *Nat Rev Mol Cell Biol* **6**, 891-898. (DOI:10.1038/nrm1742).

[3] Winner, B., Jappelli, R., Maji, S. K., Desplats, P. A., Boyer, L., Aigner, S., Hetzer, C., Loher, T., Vilar, M., Campioni, S., et al. 2011 In vivo demonstration that alpha-synuclein oligomers are toxic. *Proc Natl Acad Sci U S A* **108**, 4194-4199. (DOI:10.1073/pnas.1100976108).

[4] Walsh, D. M., Klyubin, I., Fadeeva, J. V., Cullen, W. K., Anwyl, R., Wolfe, M. S., Rowan, M. J. & Selkoe, D. J. 2002 Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation in vivo. *Nature* **416**, 535-539. (DOI:10.1038/416535a).

[5] Yerbury, J. J., Ooi, L., Dillin, A., Saunders, D. N., Hatters, D. M., Beart, P. M., Cashman, N. R., Wilson, M. R. & Ecroyd, H. 2016 Walking the tightrope: proteostasis

and neurodegenerative disease. *J Neurochem* **137**, 489-505. (DOI:10.1111/jnc.13575).

[6] Balch, W. E., Morimoto, R. I., Dillin, A. & Kelly, J. W. 2008 Adapting proteostasis for disease intervention. *Science* **319**, 916-919. (DOI:10.1126/science.1141448).

[7] Muchowski, P. J. & Wacker, J. L. 2005 Modulation of neurodegeneration by molecular chaperones. *Nat Rev Neurosci* **6**, 11-22. (DOI:10.1038/nrn1587).

[8] Hetz, C., Chevet, E. & Oakes, S. A. 2015 Proteostasis control by the unfolded protein response. *Nat Cell Biol* **17**, 829-838. (DOI:10.1038/ncb3184).

[9] Ravikumar, B., Sarkar, S., Davies, J. E., Futter, M., Garcia-Arencibia, M., Green-Thompson, Z. W., Jimenez-Sanchez, M., Korolchuk, V. I., Lichtenberg, M., Luo, S., et al. 2010 Regulation of mammalian autophagy in physiology and pathophysiology. *Physiol Rev* **90**, 1383-1435. (DOI:10.1152/physrev.00030.2009).

[10] Diaz-Villanueva, J. F., Diaz-Molina, R. & Garcia-Gonzalez, V. 2015 Protein Folding and Mechanisms of Proteostasis. *Int J Mol Sci* **16**, 17193-17230. (DOI:10.3390/ijms160817193).

[11] Tanaka, K. & Matsuda, N. 2014 Proteostasis and neurodegeneration: the roles of proteasomal degradation and autophagy. *Biochim Biophys Acta* **1843**, 197-204. (DOI:10.1016/j.bbamcr.2013.03.012).

[12] Hartl, F. U., Bracher, A. & Hayer-Hartl, M. 2011 Molecular chaperones in protein folding and proteostasis. *Nature* **475**, 324-332. (DOI:10.1038/nature10317).

[13] Tsai, J. & Douglas, M. G. 1996 A conserved HPD sequence of the J-domain is necessary for YDJ1 stimulation of Hsp70 ATPase activity at a site distinct from substrate binding. *J Biol Chem* **271**, 9347-9354.

[14] Kampinga, H. H., Hageman, J., Vos, M. J., Kubota, H., Tanguay, R. M., Bruford, E. A., Cheetham, M. E., Chen, B. & Hightower, L. E. 2009 Guidelines for the nomenclature of the human heat shock proteins. *Cell Stress Chaperones* **14**, 105-111. (DOI:10.1007/s12192-008-0068-7).

[15] Craig, E. A. & Marszalek, J. 2017 How Do J-Proteins Get Hsp70 to Do So Many Different Things? *Trends Biochem Sci*. (DOI:10.1016/j.tibs.2017.02.007).

[16] Cheetham, M. E. & Caplan, A. J. 1998 Structure, function and evolution of DnaJ: conservation and adaptation of chaperone function. *Cell Stress Chaperones* **3**, 28-36.

[17] Kampinga, H. H. & Craig, E. A. 2010 The HSP70 chaperone machinery: J proteins as drivers of functional specificity. *Nat Rev Mol Cell Biol* **11**, 579-592. (DOI:10.1038/nrm2941).

[18] Macario, A. J. & Conway de Macario, E. 2007 Chaperonopathies and chaperonotherapy. *FEBS Lett* **581**, 3681-3688. (DOI:10.1016/j.febslet.2007.04.030).

[19] Smith, H. L., Li, W. & Cheetham, M. E. 2015 Molecular chaperones and neuronal proteostasis. *Semin Cell Dev Biol* **40**, 142-152. (DOI:10.1016/j.semcdb.2015.03.003).
[20] Koutras, C. & Braun, J. E. 2014 J protein mutations and resulting proteostasis collapse. *Front Cell Neurosci* **8**, 191. (DOI:10.3389/fncel.2014.00191).
[21] El Khouri, E., Thomas, L., Jeanson, L., Bequignon, E., Vallette, B., Duquesnoy, P., Montantin, G., Copin, B., Dastot-Le Moal, F., Blanchon, S., et al. 2016 Mutations in DNAJB13, Encoding an HSP40 Family Member, Cause Primary Ciliary Dyskinesia and Male Infertility. *Am J Hum Genet* **99**, 489-500. (DOI:10.1016/j.ajhg.2016.06.022).
[22] Anikster, Y., Haack, T. B., Vilboux, T., Pode-Shakked, B., Thony, B., Shen, N., Guarani, V., Meissner, T., Mayatepek, E., Trefz, F. K., et al. 2017 Biallelic Mutations in DNAJC12 Cause Hyperphenylalaninemia, Dystonia, and Intellectual Disability. *Am J Hum Genet* **100**, 257-266. (DOI:10.1016/j.ajhg.2017.01.002).
[23] Tummala, H., Walne, A. J., Williams, M., Bockett, N., Collopy, L., Cardoso, S., Ellison, A., Wynn, R., Leblanc, T., Fitzgibbon, J., et al. 2016 DNAJC21 Mutations Link a Cancer-Prone Bone Marrow Failure Syndrome to Corruption in 60S Ribosome Subunit Maturation. *Am J Hum Genet* **99**, 115-124. (DOI:10.1016/j.ajhg.2016.05.002).
[24] Chapple, J. P. & Cheetham, M. E. 2003 The chaperone environment at the cytoplasmic face of the endoplasmic reticulum can modulate rhodopsin processing and inclusion formation. *J Biol Chem* **278**, 19087-19094. (DOI:10.1074/jbc.M212349200).
[25] Westhoff, B., Chapple, J. P., van der Spuy, J., Hohfeld, J. & Cheetham, M. E. 2005 HSJ1 is a neuronal shuttling factor for the sorting of chaperone clients to the proteasome. *Curr Biol* **15**, 1058-1064. (DOI:10.1016/j.cub.2005.04.058).
[26] Tazir, M., Hamadouche, T., Nouioua, S., Mathis, S. & Vallat, J. M. 2014 Hereditary motor and sensory neuropathies or Charcot-Marie-Tooth diseases: an update. *J Neurol Sci* **347**, 14-22. (DOI:10.1016/j.jns.2014.10.013).
[27] Schabhuttl, M., Wieland, T., Senderek, J., Baets, J., Timmerman, V., De Jonghe, P., Reilly, M. M., Stieglbauer, K., Laich, E., Windhager, R., et al. 2014 Whole-exome sequencing in patients with inherited neuropathies: outcome and challenges. *J Neurol* **261**, 970-982. (DOI:10.1007/s00415-014-7289-8).
[28] Gess, B., Auer-Grumbach, M., Schirmacher, A., Strom, T., Zitzelsberger, M., Rudnik-Schoneborn, S., Rohr, D., Halfter, H., Young, P. & Senderek, J. 2014 HSJ1-related hereditary neuropathies: novel mutations and extended clinical spectrum. *Neurology* **83**, 1726-1732. (DOI:10.1212/WNL.0000000000000966).

[29] Rossor, A. M., Kalmar, B., Greensmith, L. & Reilly, M. M. 2012 The distal hereditary motor neuropathies. *J Neurol Neurosurg Psychiatry* **83**, 6-14. (DOI:10.1136/jnnp-2011-300952).

[30] Bansagi, B., Griffin, H., Whittaker, R. G., Antoniadi, T., Evangelista, T., Miller, J., Greenslade, M., Forester, N., Duff, J., Bradshaw, A., et al. 2017 Genetic heterogeneity of motor neuropathies. *Neurology*. (DOI:10.1212/WNL.0000000000003772).

[31] Blumen, S. C., Astord, S., Robin, V., Vignaud, L., Toumi, N., Cieslik, A., Achiron, A., Carasso, R. L., Gurevich, M., Braverman, I., et al. 2012 A rare recessive distal hereditary motor neuropathy with HSJ1 chaperone mutation. *Ann Neurol* **71**, 509-519. (DOI:10.1002/ana.22684).

[32] Frasquet, M., Chumillas, M. J., Vilchez, J. J., Marquez-Infante, C., Palau, F., Vazquez-Costa, J. F., Lupo, V., Espinos, C. & Sevilla, T. 2016 Phenotype and natural history of inherited neuropathies caused by HSJ1 c.352+1G>A mutation. *J Neurol Neurosurg Psychiatry* **87**, 1265-1268. (DOI:10.1136/jnnp-2015-312890).

[33] Lupo, V., Garcia-Garcia, F., Sancho, P., Tello, C., Garcia-Romero, M., Villarreal, L., Alberti, A., Sivera, R., Dopazo, J., Pascual-Pascual, S. I., et al. 2016 Assessment of Targeted Next-Generation Sequencing as a Tool for the Diagnosis of Charcot-Marie-Tooth Disease and Hereditary Motor Neuropathy. *J Mol Diagn* **18**, 225-234. (DOI:10.1016/j.jmoldx.2015.10.005).

[34] Sanchez, E., Darvish, H., Mesias, R., Taghavi, S., Firouzabadi, S. G., Walker, R. H., Tafakhori, A. & Paisan-Ruiz, C. 2016 Identification of a Large DNAJB2 Deletion in a Family with Spinal Muscular Atrophy and Parkinsonism. *Hum Mutat* **37**, 1180-1189. (DOI:10.1002/humu.23055).

[35] Gonzaga-Jauregui, C., Harel, T., Gambin, T., Kousi, M., Griffin, L. B., Francescatto, L., Ozes, B., Karaca, E., Jhangiani, S. N., Bainbridge, M. N., et al. 2015 Exome Sequence Analysis Suggests that Genetic Burden Contributes to Phenotypic Variability and Complex Neuropathy. *Cell Rep* **12**, 1169-1183. (DOI:10.1016/j.celrep.2015.07.023).

[36] Chen, M. S., Roti, J. R. & Laszlo, A. 1999 Hsc40, a new member of the hsp40 family, exhibits similar expression profile to that of hsc70 in mammalian cells. *Gene* **238**, 333-341.

[37] Hageman, J., van Waarde, M. A., Zylicz, A., Walerych, D. & Kampinga, H. H. 2011 The diverse members of the mammalian HSP70 machine show distinct chaperone-like activities. *Biochem J* **435**, 127-142. (DOI:10.1042/bj20101247).

[38] Chuang, J. Z., Zhou, H., Zhu, M., Li, S. H., Li, X. J. & Sung, C. H. 2002 Characterization of a brain-enriched chaperone, MRJ, that inhibits Huntingtin

aggregation and toxicity independently. *J Biol Chem* **277**, 19831-19838. (DOI:10.1074/jbc.M109613200).

[39] Mitra, A., Fillmore, R. A., Metge, B. J., Rajesh, M., Xi, Y., King, J., Ju, J., Pannell, L., Shevde, L. A. & Samant, R. S. 2008 Large isoform of MRJ (DNAJB6) reduces malignant activity of breast cancer. *Breast Cancer Res* **10**, R22. (DOI:10.1186/bcr1874).

[40] Yabe, I., Tanino, M., Yaguchi, H., Takiyama, A., Cai, H., Kanno, H., Takahashi, I., Hayashi, Y. K., Watanabe, M., Takahashi, H., et al. 2014 Pathology of frontotemporal dementia with limb girdle muscular dystrophy caused by a DNAJB6 mutation. *Clin Neurol Neurosurg* **127**, 10-12. (DOI:10.1016/j.clineuro.2014.09.013).

[41] Ruggieri, A., Saredi, S., Zanotti, S., Pasanisi, M. B., Maggi, L. & Mora, M. 2016 DNAJB6 Myopathies: Focused Review on an Emerging and Expanding Group of Myopathies. *Front Mol Biosci* **3**, 63. (DOI:10.3389/fmolb.2016.00063).

[42] Sandell, S., Huovinen, S., Palmio, J., Raheem, O., Lindfors, M., Zhao, F., Haapasalo, H. & Udd, B. 2016 Diagnostically important muscle pathology in DNAJB6 mutated LGMD1D. *Acta Neuropathol Commun* **4**, 9. (DOI:10.1186/s40478-016-0276-9).

[43] Sato, T., Hayashi, Y. K., Oya, Y., Kondo, T., Sugie, K., Kaneda, D., Houzen, H., Yabe, I., Sasaki, H., Noguchi, S., et al. 2013 DNAJB6 myopathy in an Asian cohort and cytoplasmic/nuclear inclusions. *Neuromuscul Disord* **23**, 269-276. (DOI:10.1016/j.nmd.2012.12.010).

[44] Bengoechea, R., Pittman, S. K., Tuck, E. P., True, H. L. & Weihl, C. C. 2015 Myofibrillar disruption and RNA-binding protein aggregation in a mouse model of limb-girdle muscular dystrophy 1D. *Hum Mol Genet* **24**, 6588-6602. (DOI:10.1093/hmg/ddv363).

[45] Li, S., Zhang, P., Freibaum, B. D., Kim, N. C., Kolaitis, R. M., Molliex, A., Kanagaraj, A. P., Yabe, I., Tanino, M., Tanaka, S., et al. 2016 Genetic interaction of hnRNPA2B1 and DNAJB6 in a Drosophila model of multisystem proteinopathy. *Hum Mol Genet* **25**, 936-950. (DOI:10.1093/hmg/ddv627).

[46] Melville, M. W., Tan, S. L., Wambach, M., Song, J., Morimoto, R. I. & Katze, M. G. 1999 The cellular inhibitor of the PKR protein kinase, P58(IPK), is an influenza virus-activated co-chaperone that modulates heat shock protein 70 activity. *J Biol Chem* **274**, 3797-3803.

[47] Tao, J., Wu, Y., Ron, D. & Sha, B. 2008 Preliminary X-ray crystallographic studies of mouse UPR responsive protein P58(IPK) TPR fragment. *Acta crystallographica. Section F, Structural biology and crystallization communications* **64**, 108-110. (DOI:10.1107/s1744309108000833).

[48] Yan, W., Frank, C. L., Korth, M. J., Sopher, B. L., Novoa, I., Ron, D. & Katze, M. G. 2002 Control of PERK eIF2alpha kinase activity by the endoplasmic reticulum stress-induced molecular chaperone P58IPK. *Proc Natl Acad Sci U S A* **99**, 15920-15925. (DOI:10.1073/pnas.252341799).

[49] Rutkowski, D. T., Kang, S. W., Goodman, A. G., Garrison, J. L., Taunton, J., Katze, M. G., Kaufman, R. J. & Hegde, R. S. 2007 The role of p58IPK in protecting the stressed endoplasmic reticulum. *Mol Biol Cell* **18**, 3681-3691. (DOI:10.1091/mbc.E07-03-0272).

[50] Oyadomari, S., Yun, C., Fisher, E. A., Kreglinger, N., Kreibich, G., Oyadomari, M., Harding, H. P., Goodman, A. G., Harant, H., Garrison, J. L., et al. 2006 Cotranslocational degradation protects the stressed endoplasmic reticulum from protein overload. *Cell* **126**, 727-739. (DOI:10.1016/j.cell.2006.06.051).

[51] Petrova, K., Oyadomari, S., Hendershot, L. M. & Ron, D. 2008 Regulated association of misfolded endoplasmic reticulum lumenal proteins with P58/DNAJc3. *EMBO J* **27**, 2862-2872. (DOI:10.1038/emboj.2008.199).

[52] Ladiges, W. C., Knoblaugh, S. E., Morton, J. F., Korth, M. J., Sopher, B. L., Baskin, C. R., MacAuley, A., Goodman, A. G., LeBoeuf, R. C. & Katze, M. G. 2005 Pancreatic beta-cell failure and diabetes in mice with a deletion mutation of the endoplasmic reticulum molecular chaperone gene P58IPK. *Diabetes* **54**, 1074-1081.

[53] Synofzik, M., Haack, T. B., Kopajtich, R., Gorza, M., Rapaport, D., Greiner, M., Schonfeld, C., Freiberg, C., Schorr, S., Holl, R. W., et al. 2014 Absence of BiP co-chaperone DNAJC3 causes diabetes mellitus and multisystemic neurodegeneration. *Am J Hum Genet* **95**, 689-697. (DOI:10.1016/j.ajhg.2014.10.013).

[54] Tawo, R., Pokrzywa, W., Kevei, E., Akyuz, M. E., Balaji, V., Adrian, S., Hohfeld, J. & Hoppe, T. 2017 The Ubiquitin Ligase CHIP Integrates Proteostasis and Aging by Regulation of Insulin Receptor Turnover. *Cell* **169**, 470-482 e413. (DOI:10.1016/j.cell.2017.04.003).

[55] Fernandez-Chacon, R., Wolfel, M., Nishimune, H., Tabares, L., Schmitz, F., Castellano-Munoz, M., Rosenmund, C., Montesinos, M. L., Sanes, J. R., Schneggenburger, R., et al. 2004 The synaptic vesicle protein CSP alpha prevents presynaptic degeneration. *Neuron* **42**, 237-251.

[56] Greaves, J. & Chamberlain, L. H. 2006 Dual role of the cysteine-string domain in membrane binding and palmitoylation-dependent sorting of the molecular chaperone cysteine-string protein. *Mol Biol Cell* **17**, 4748-4759. (DOI:10.1091/mbc.E06-03-0183).

[57] Zhang, Y. Q., Henderson, M. X., Colangelo, C. M., Ginsberg, S. D., Bruce, C., Wu, T. & Chandra, S. S. 2012 Identification of CSPalpha clients reveals a role in dynamin 1 regulation. *Neuron* **74**, 136-150. (DOI:10.1016/j.neuron.2012.01.029).

[58] Chamberlain, L. H., Graham, M. E., Kane, S., Jackson, J. L., Maier, V. H., Burgoyne, R. D. & Gould, G. W. 2001 The synaptic vesicle protein, cysteine-string protein, is associated with the plasma membrane in 3T3-L1 adipocytes and interacts with syntaxin 4. *J Cell Sci* **114**, 445-455.

[59] Evans, G. J. & Morgan, A. 2002 Phosphorylation-dependent interaction of the synaptic vesicle proteins cysteine string protein and synaptotagmin I. *Biochem J* **364**, 343-347. (DOI:10.1042/BJ20020123).

[60] Tobaben, S., Thakur, P., Fernandez-Chacon, R., Sudhof, T. C., Rettig, J. & Stahl, B. 2001 A trimeric protein complex functions as a synaptic chaperone machine. *Neuron* **31**, 987-999.

[61] Rozas, J. L., Gomez-Sanchez, L., Mircheski, J., Linares-Clemente, P., Nieto-Gonzalez, J. L., Vazquez, M. E., Lujan, R. & Fernandez-Chacon, R. 2012 Motorneurons require cysteine string protein-alpha to maintain the readily releasable vesicular pool and synaptic vesicle recycling. *Neuron* **74**, 151-165. (DOI:10.1016/j.neuron.2012.02.019).

[62] Burgoyne, R. D. & Morgan, A. 2015 Cysteine string protein (CSP) and its role in preventing neurodegeneration. *Semin Cell Dev Biol* **40**, 153-159. (DOI:10.1016/j.semcdb.2015.03.008).

[63] Benitez, B. A., Cairns, N. J., Schmidt, R. E., Morris, J. C., Norton, J. B., Cruchaga, C. & Sands, M. S. 2015 Clinically early-stage CSPalpha mutation carrier exhibits remarkable terminal stage neuronal pathology with minimal evidence of synaptic loss. *Acta Neuropathol Commun* **3**, 73. (DOI:10.1186/s40478-015-0256-5).

[64] Benitez, B. A., Alvarado, D., Cai, Y., Mayo, K., Chakraverty, S., Norton, J., Morris, J. C., Sands, M. S., Goate, A. & Cruchaga, C. 2011 Exome-sequencing confirms DNAJC5 mutations as cause of adult neuronal ceroid-lipofuscinosis. *PLoS One* **6**, e26741. (DOI:10.1371/journal.pone.0026741).

[65] Noskova, L., Stranecky, V., Hartmannova, H., Pristoupilova, A., Baresova, V., Ivanek, R., Hulkova, H., Jahnova, H., van der Zee, J., Staropoli, J. F., et al. 2011 Mutations in DNAJC5, encoding cysteine-string protein alpha, cause autosomal-dominant adult-onset neuronal ceroid lipofuscinosis. *Am J Hum Genet* **89**, 241-252. (DOI:10.1016/j.ajhg.2011.07.003).

[66] Cadieux-Dion, M., Andermann, E., Lachance-Touchette, P., Ansorge, O., Meloche, C., Barnabe, A., Kuzniecky, R. I., Andermann, F., Faught, E., Leonberg, S.,

et al. 2013 Recurrent mutations in DNAJC5 cause autosomal dominant Kufs disease. *Clin Genet* **83**, 571-575. (DOI:10.1111/cge.12020).

[67] Velinov, M., Dolzhanskaya, N., Gonzalez, M., Powell, E., Konidari, I., Hulme, W., Staropoli, J. F., Xin, W., Wen, G. Y., Barone, R., et al. 2012 Mutations in the gene DNAJC5 cause autosomal dominant Kufs disease in a proportion of cases: study of the Parry family and 8 other families. *PLoS One* **7**, e29729. (DOI:10.1371/journal.pone.0029729).

[68] Diez-Ardanuy, C., Greaves, J., Munro, K. R., Tomkinson, N. C. & Chamberlain, L. H. 2017 A cluster of palmitoylated cysteines are essential for aggregation of cysteine-string protein mutants that cause neuronal ceroid lipofuscinosis. *Sci Rep* **7**, 10. (DOI:10.1038/s41598-017-00036-8).

[69] Greaves, J., Lemonidis, K., Gorleku, O. A., Cruchaga, C., Grefen, C. & Chamberlain, L. H. 2012 Palmitoylation-induced aggregation of cysteine-string protein mutants that cause neuronal ceroid lipofuscinosis. *J Biol Chem* **287**, 37330-37339. (DOI:10.1074/jbc.M112.389098).

[70] Henderson, M. X., Wirak, G. S., Zhang, Y. Q., Dai, F., Ginsberg, S. D., Dolzhanskaya, N., Staropoli, J. F., Nijssen, P. C., Lam, T. T., Roth, A. F., et al. 2016 Neuronal ceroid lipofuscinosis with DNAJC5/CSPalpha mutation has PPT1 pathology and exhibit aberrant protein palmitoylation. *Acta Neuropathol* **131**, 621-637. (DOI:10.1007/s00401-015-1512-2).

[71] Kashyap, S. S., Johnson, J. R., McCue, H. V., Chen, X., Edmonds, M. J., Ayala, M., Graham, M. E., Jenn, R. C., Barclay, J. W., Burgoyne, R. D., et al. 2014 Caenorhabditis elegans dnj-14, the orthologue of the DNAJC5 gene mutated in adult onset neuronal ceroid lipofuscinosis, provides a new platform for neuroprotective drug screening and identifies a SIR-2.1-independent action of resveratrol. *Hum Mol Genet* **23**, 5916-5927. (DOI:10.1093/hmg/ddu316).

[72] Ahle, S. & Ungewickell, E. 1990 Auxilin, a newly identified clathrin-associated protein in coated vesicles from bovine brain. *J Cell Biol* **111**, 19-29.

[73] Fotin, A., Cheng, Y., Grigorieff, N., Walz, T., Harrison, S. C. & Kirchhausen, T. 2004 Structure of an auxilin-bound clathrin coat and its implications for the mechanism of uncoating. *Nature* **432**, 649-653. (DOI:10.1038/nature03078).

[74] Bocking, T., Aguet, F., Harrison, S. C. & Kirchhausen, T. 2011 Single-molecule analysis of a molecular disassemblase reveals the mechanism of Hsc70-driven clathrin uncoating. *Nat Struct Mol Biol* **18**, 295-301. (DOI:10.1038/nsmb.1985).

[75] Holstein, S. E., Ungewickell, H. & Ungewickell, E. 1996 Mechanism of clathrin basket dissociation: separate functions of protein domains of the DnaJ homologue auxilin. *J Cell Biol* **135**, 925-937.

[76] Massol, R. H., Boll, W., Griffin, A. M. & Kirchhausen, T. 2006 A burst of auxilin recruitment determines the onset of clathrin-coated vesicle uncoating. *Proc Natl Acad Sci U S A* **103**, 10265-10270. (DOI:10.1073/pnas.0603369103).

[77] Edvardson, S., Cinnamon, Y., Ta-Shma, A., Shaag, A., Yim, Y. I., Zenvirt, S., Jalas, C., Lesage, S., Brice, A., Taraboulos, A., et al. 2012 A deleterious mutation in DNAJC6 encoding the neuronal-specific clathrin-uncoating co-chaperone auxilin, is associated with juvenile parkinsonism. *PLoS One* **7**, e36458. (DOI:10.1371/journal.pone.0036458).

[78] Koroglu, C., Baysal, L., Cetinkaya, M., Karasoy, H. & Tolun, A. 2013 DNAJC6 is responsible for juvenile parkinsonism with phenotypic variability. *Parkinsonism Relat Disord* **19**, 320-324. (DOI:10.1016/j.parkreldis.2012.11.006).

[79] Vauthier, V., Jaillard, S., Journel, H., Dubourg, C., Jockers, R. & Dam, J. 2012 Homozygous deletion of an 80 kb region comprising part of DNAJC6 and LEPR genes on chromosome 1P31.3 is associated with early onset obesity, mental retardation and epilepsy. *Mol Genet Metab* **106**, 345-350. (DOI:10.1016/j.ymgme.2012.04.026).

[80] Olgiati, S., Quadri, M., Fang, M., Rood, J. P., Saute, J. A., Chien, H. F., Bouwkamp, C. G., Graafland, J., Minneboo, M., Breedveld, G. J., et al. 2016 DNAJC6 Mutations Associated With Early-Onset Parkinson's Disease. *Ann Neurol* **79**, 244-256. (DOI:10.1002/ana.24553).

[81] Katoh, M. & Katoh, M. 2003 Identification and characterization of FLJ10737 and CAMTA1 genes on the commonly deleted region of neuroblastoma at human chromosome 1p36.31-p36.23. *Int J Oncol* **23**, 1219-1224.

[82] Henrich, K. O., Claas, A., Praml, C., Benner, A., Mollenhauer, J., Poustka, A., Schwab, M. & Westermann, F. 2007 Allelic variants of CAMTA1 and FLJ10737 within a commonly deleted region at 1p36 in neuroblastoma. *Eur J Cancer* **43**, 607-616. (DOI:10.1016/j.ejca.2006.09.023).

[83] Xie, J., Marusich, M. F., Souda, P., Whitelegge, J. & Capaldi, R. A. 2007 The mitochondrial inner membrane protein mitofilin exists as a complex with SAM50, metaxins 1 and 2, coiled-coil-helix coiled-coil-helix domain-containing protein 3 and 6 and DnaJC11. *FEBS Lett* **581**, 3545-3549. (DOI:10.1016/j.febslet.2007.06.052).

[84] Andrews, B., Carroll, J., Ding, S., Fearnley, I. M. & Walker, J. E. 2013 Assembly factors for the membrane arm of human complex I. *Proc Natl Acad Sci U S A* **110**, 18934-18939. (DOI:10.1073/pnas.1319247110).

[85] Ioakeimidis, F., Ott, C., Kozjak-Pavlovic, V., Violitzi, F., Rinotas, V., Makrinou, E., Eliopoulos, E., Fasseas, C., Kollias, G. & Douni, E. 2014 A splicing mutation in the novel mitochondrial protein DNAJC11 causes motor neuron pathology associated

with cristae disorganization, and lymphoid abnormalities in mice. *PLoS One* **9**, e104237. (DOI:10.1371/journal.pone.0104237).

[86] Girard, M., Poupon, V., Blondeau, F. & McPherson, P. S. 2005 The DnaJ-domain protein RME-8 functions in endosomal trafficking. *J Biol Chem* **280**, 40135-40143. (DOI:10.1074/jbc.M505036200).

[87] Popoff, V., Mardones, G. A., Bai, S. K., Chambon, V., Tenza, D., Burgos, P. V., Shi, A., Benaroch, P., Urbe, S., Lamaze, C., et al. 2009 Analysis of articulation between clathrin and retromer in retrograde sorting on early endosomes. *Traffic* **10**, 1868-1880. (DOI:10.1111/j.1600-0854.2009.00993.x).

[88] Vilarino-Guell, C., Rajput, A., Milnerwood, A. J., Shah, B., Szu-Tu, C., Trinh, J., Yu, I., Encarnacion, M., Munsie, L. N., Tapia, L., et al. 2014 DNAJC13 mutations in Parkinson disease. *Hum Mol Genet* **23**, 1794-1801. (DOI:10.1093/hmg/ddt570).

[89] Deng, H. X., Shi, Y., Yang, Y., Ahmeti, K. B., Miller, N., Huang, C., Cheng, L., Zhai, H., Deng, S., Nuytemans, K., et al. 2016 Identification of TMEM230 mutations in familial Parkinson's disease. *Nat Genet* **48**, 733-739. (DOI:10.1038/ng.3589).

[90] Lorenzo-Betancor, O., Ogaki, K., Soto-Ortolaza, A. I., Labbe, C., Walton, R. L., Strongosky, A. J., van Gerpen, J. A., Uitti, R. J., McLean, P. J., Springer, W., et al. 2015 DNAJC13 p.Asn855Ser mutation screening in Parkinson's disease and pathologically confirmed Lewy body disease patients. *Eur J Neurol* **22**, 1323-1325. (DOI:10.1111/ene.12770).

[91] Gustavsson, E. K., Trinh, J., Guella, I., Vilarino-Guell, C., Appel-Cresswell, S., Stoessl, A. J., Tsui, J. K., McKeown, M., Rajput, A., Rajput, A. H., et al. 2015 DNAJC13 genetic variants in parkinsonism. *Mov Disord* **30**, 273-278. (DOI:10.1002/mds.26064).

[92] Ross, J. P., Dupre, N., Dauvilliers, Y., Strong, S., Ambalavanan, A., Spiegelman, D., Dionne-Laporte, A., Pourcher, E., Langlois, M., Boivin, M., et al. 2016 Analysis of DNAJC13 mutations in French-Canadian/French cohort of Parkinson's disease. *Neurobiol Aging* **45**, 212 e213-217. (DOI:10.1016/j.neurobiolaging.2016.04.023).

[93] Mokranjac, D., Sichting, M., Neupert, W. & Hell, K. 2003 Tim14, a novel key component of the import motor of the TIM23 protein translocase of mitochondria. *EMBO J* **22**, 4945-4956. (DOI:10.1093/emboj/cdg485).

[94] Davey, K. M., Parboosingh, J. S., McLeod, D. R., Chan, A., Casey, R., Ferreira, P., Snyder, F. F., Bridge, P. J. & Bernier, F. P. 2006 Mutation of DNAJC19, a human homologue of yeast inner mitochondrial membrane co-chaperones, causes DCMA syndrome, a novel autosomal recessive Barth syndrome-like condition. *J Med Genet* **43**, 385-393. (DOI:10.1136/jmg.2005.036657).

[95] Ojala, T., Polinati, P., Manninen, T., Hiippala, A., Rajantie, J., Karikoski, R., Suomalainen, A. & Tyni, T. 2012 New mutation of mitochondrial DNAJC19 causing dilated and noncompaction cardiomyopathy, anemia, ataxia, and male genital anomalies. *Pediatr Res* **72**, 432-437. (DOI:10.1038/pr.2012.92).

[96] Al Teneiji, A., Siriwardena, K., George, K., Mital, S. & Mercimek-Mahmutoglu, S. 2016 Progressive Cerebellar Atrophy and a Novel Homozygous Pathogenic DNAJC19 Variant as a Cause of Dilated Cardiomyopathy Ataxia Syndrome. *Pediatr Neurol* **62**, 58-61. (DOI:10.1016/j.pediatrneurol.2016.03.020).

[97] Ucar, S. K., Mayr, J. A., Feichtinger, R. G., Canda, E., Coker, M. & Wortmann, S. B. 2016 Previously Unreported Biallelic Mutation in DNAJC19: Are Sensorineural Hearing Loss and Basal Ganglia Lesions Additional Features of Dilated Cardiomyopathy and Ataxia (DCMA) Syndrome? *JIMD Rep*. (DOI:10.1007/8904_2016_23).

[98] Parfitt, D. A., Michael, G. J., Vermeulen, E. G., Prodromou, N. V., Webb, T. R., Gallo, J. M., Cheetham, M. E., Nicoll, W. S., Blatch, G. L. & Chapple, J. P. 2009 The ataxia protein sacsin is a functional co-chaperone that protects against polyglutamine-expanded ataxin-1. *Hum Mol Genet* **18**, 1556-1565. (DOI:10.1093/hmg/ddp067).

[99] Anderson, J. F., Siller, E. & Barral, J. M. 2010 The sacsin repeating region (SRR): a novel Hsp90-related supra-domain associated with neurodegeneration. *J Mol Biol* **400**, 665-674. (DOI:10.1016/j.jmb.2010.05.023).

[100] Girard, M., Lariviere, R., Parfitt, D. A., Deane, E. C., Gaudet, R., Nossova, N., Blondeau, F., Prenosil, G., Vermeulen, E. G., Duchen, M. R., et al. 2012 Mitochondrial dysfunction and Purkinje cell loss in autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS). *Proc Natl Acad Sci U S A* **109**, 1661-1666. (DOI:10.1073/pnas.1113166109).

[101] Bouchard, J. P., Barbeau, A., Bouchard, R. & Bouchard, R. W. 1978 Autosomal recessive spastic ataxia of Charlevoix-Saguenay. *Can J Neurol Sci* **5**, 61-69.

[102] Engert, J. C., Berube, P., Mercier, J., Dore, C., Lepage, P., Ge, B., Bouchard, J. P., Mathieu, J., Melancon, S. B., Schalling, M., et al. 2000 ARSACS, a spastic ataxia common in northeastern Quebec, is caused by mutations in a new gene encoding an 11.5-kb ORF. *Nat Genet* **24**, 120-125. (DOI:10.1038/72769).

[103] Thiffault, I., Dicaire, M. J., Tetreault, M., Huang, K. N., Demers-Lamarche, J., Bernard, G., Duquette, A., Lariviere, R., Gehring, K., Montpetit, A., et al. 2013 Diversity of ARSACS mutations in French-Canadians. *Can J Neurol Sci* **40**, 61-66.

[104] Breckpot, J., Takiyama, Y., Thienpont, B., Van Vooren, S., Vermeesch, J. R., Ortibus, E. & Devriendt, K. 2008 A novel genomic disorder: a deletion of the SACS

gene leading to spastic ataxia of Charlevoix-Saguenay. *Eur J Hum Genet* **16**, 1050-1054. (DOI:10.1038/ejhg.2008.58).

[105] Vermeer, S., Meijer, R. P., Pijl, B. J., Timmermans, J., Cruysberg, J. R., Bos, M. M., Schelhaas, H. J., van de Warrenburg, B. P., Knoers, N. V., Scheffer, H., et al. 2008 ARSACS in the Dutch population: a frequent cause of early-onset cerebellar ataxia. *Neurogenetics* **9**, 207-214. (DOI:10.1007/s10048-008-0131-7).

[106] Baets, J., Deconinck, T., Smets, K., Goossens, D., Van den Bergh, P., Dahan, K., Schmedding, E., Santens, P., Rasic, V. M., Van Damme, P., et al. 2010 Mutations in SACS cause atypical and late-onset forms of ARSACS. *Neurology* **75**, 1181-1188. (DOI:10.1212/WNL.0b013e3181f4d86c).

[107] Lariviere, R., Gaudet, R., Gentil, B. J., Girard, M., Conte, T. C., Minotti, S., Leclerc-Desaulniers, K., Gehring, K., McKinney, R. A., Shoubridge, E. A., et al. 2015 Sacs knockout mice present pathophysiological defects underlying autosomal recessive spastic ataxia of Charlevoix-Saguenay. *Hum Mol Genet* **24**, 727-739. (DOI:10.1093/hmg/ddu491).

[108] Bradshaw, T. Y., Romano, L. E., Duncan, E. J., Nethisinghe, S., Abeti, R., Michael, G. J., Giunti, P., Vermeer, S. & Chapple, J. P. 2016 A reduction in Drp1-mediated fission compromises mitochondrial health in autosomal recessive spastic ataxia of Charlevoix Saguenay. *Hum Mol Genet* **25**, 3232-3244. (DOI:10.1093/hmg/ddw173).

[109] Shao, J. & Diamond, M. I. 2007 Polyglutamine diseases: emerging concepts in pathogenesis and therapy. *Hum Mol Genet* **16 Spec No. 2**, R115-123. (DOI:10.1093/hmg/ddm213).

[110] Cummings, C. J., Mancini, M. A., Antalffy, B., DeFranco, D. B., Orr, H. T. & Zoghbi, H. Y. 1998 Chaperone suppression of aggregation and altered subcellular proteasome localization imply protein misfolding in SCA1. *Nat Genet* **19**, 148-154.

[111] Gao, X.-C., Zhou, C.-J., Zhou, Z.-R., Zhang, Y.-H., Zheng, X.-M., Song, A.-X. & Hu, H.-Y. 2011 Co-Chaperone HSJ1a Dually Regulates the Proteasomal Degradation of Ataxin-3. *PLOS ONE* **6**, e19763. (DOI:10.1371/journal.pone.0019763).

[112] Wyttenbach, A., Carmichael, J., Swartz, J., Furlong, R. A., Narain, Y., Rankin, J. & Rubinsztein, D. C. 2000 Effects of heat shock, heat shock protein 40 (HDJ-2), and proteasome inhibition on protein aggregation in cellular models of Huntington's disease. *Proceedings of the National Academy of Sciences of the United States of America* **97**, 2898-2903.

[113] Ormsby, A. R., Ramdzan, Y. M., Mok, Y. F., Jovanoski, K. D. & Hatters, D. M. 2013 A platform to view huntingtin exon 1 aggregation flux in the cell reveals

divergent influences from chaperones hsp40 and hsp70. *J Biol Chem* **288**, 37192-37203. (DOI:10.1074/jbc.M113.486944).

[114] Jana, N. R., Tanaka, M., Wang, G. & Nukina, N. 2000 Polyglutamine length-dependent interaction of Hsp40 and Hsp70 family chaperones with truncated N-terminal huntingtin: their role in suppression of aggregation and cellular toxicity. *Hum Mol Genet* **9**, 2009-2018.

[115] Bailey, C. K., Andriola, I. F., Kampinga, H. H. & Merry, D. E. 2002 Molecular chaperones enhance the degradation of expanded polyglutamine repeat androgen receptor in a cellular model of spinal and bulbar muscular atrophy. *Hum Mol Genet* **11**, 515-523.

[116] Kobayashi, Y., Kume, A., Li, M., Doyu, M., Hata, M., Ohtsuka, K. & Sobue, G. 2000 Chaperones Hsp70 and Hsp40 Suppress Aggregate Formation and Apoptosis in Cultured Neuronal Cells Expressing Truncated Androgen Receptor Protein with Expanded Polyglutamine Tract. *Journal of Biological Chemistry* **275**, 8772-8778. (DOI:10.1074/jbc.275.12.8772).

[117] Hageman, J., Rujano, M. A., van Waarde, M. A., Kakkar, V., Dirks, R. P., Govorukhina, N., Oosterveld-Hut, H. M., Lubsen, N. H. & Kampinga, H. H. 2010 A DNAJB chaperone subfamily with HDAC-dependent activities suppresses toxic protein aggregation. *Mol Cell* **37**, 355-369. (DOI:10.1016/j.molcel.2010.01.001).

[118] Mansson, C., Kakkar, V., Monsellier, E., Sourigues, Y., Harmark, J., Kampinga, H. H., Melki, R. & Emanuelsson, C. 2014 DNAJB6 is a peptide-binding chaperone which can suppress amyloid fibrillation of polyglutamine peptides at substoichiometric molar ratios. *Cell stress & chaperones* **19**, 227-239. (DOI:10.1007/s12192-013-0448-5).

[119] Nollen, E. A. A., Garcia, S. M., van Haaften, G., Kim, S., Chavez, A., Morimoto, R. I. & Plasterk, R. H. A. 2004 Genome-wide RNA interference screen identifies previously undescribed regulators of polyglutamine aggregation. *Proceedings of the National Academy of Sciences of the United States of America* **101**, 6403-6408. (DOI:10.1073/pnas.0307697101).

[120] Gillis, J., Schipper-Krom, S., Juenemann, K., Gruber, A., Coolen, S., van den Nieuwendijk, R., van Veen, H., Overkleeft, H., Goedhart, J., Kampinga, H. H., et al. 2013 The DNAJB6 and DNAJB8 protein chaperones prevent intracellular aggregation of polyglutamine peptides. *J Biol Chem* **288**, 17225-17237. (DOI:10.1074/jbc.M112.421685).

[121] Borrell-Pages, M., Canals, J. M., Cordelieres, F. P., Parker, J. A., Pineda, J. R., Grange, G., Bryson, E. A., Guillermier, M., Hirsch, E., Hantraye, P., et al. 2006 Cystamine and cysteamine increase brain levels of BDNF in Huntington disease via

HSJ1b and transglutaminase. *The Journal of clinical investigation* **116**, 1410-1424. (DOI:10.1172/jci27607).

[122] Muchowski, P. J., Schaffar, G., Sittler, A., Wanker, E. E., Hayer-Hartl, M. K. & Hartl, F. U. 2000 Hsp70 and hsp40 chaperones can inhibit self-assembly of polyglutamine proteins into amyloid-like fibrils. *Proceedings of the National Academy of Sciences of the United States of America* **97**, 7841-7846. (DOI:10.1073/pnas.140202897).

[123] Kazemi-Esfarjani, P. & Benzer, S. 2000 Genetic Suppression of Polyglutamine Toxicity in Drosophila. *Science* **287**, 1837-1840.

[124] Kuo, Y., Ren, S., Lao, U., Edgar, B. A. & Wang, T. 2013 Suppression of polyglutamine protein toxicity by co-expression of a heat-shock protein 40 and a heat-shock protein 110. *Cell Death Dis* **4**, e833. (DOI:10.1038/cddis.2013.351).

[125] Fayazi, Z., Ghosh, S., Marion, S., Bao, X., Shero, M. & Kazemi-Esfarjani, P. 2006 A Drosophila ortholog of the human MRJ modulates polyglutamine toxicity and aggregation. *Neurobiology of disease* **24**, 226-244. (DOI:10.1016/j.nbd.2006.06.015).

[126] Chan, H. Y., Warrick, J. M., Gray-Board, G. L., Paulson, H. L. & Bonini, N. M. 2000 Mechanisms of chaperone suppression of polyglutamine disease: selectivity, synergy and modulation of protein solubility in Drosophila. *Hum Mol Genet* **9**, 2811-2820.

[127] Tsou, W.-L., Hosking, R. R., Burr, A. A., Sutton, J. R., Ouyang, M., Du, X., Gomez, C. M. & Todi, S. V. 2015 DnaJ-1 and karyopherin α3 suppress degeneration in a new Drosophila model of Spinocerebellar Ataxia Type 6. *Human Molecular Genetics* **24**, 4385-4396. (DOI:10.1093/hmg/ddv174).

[128] Tsou, W.-L., Ouyang, M., Hosking, R. R., Sutton, J. R., Blount, J. R., Burr, A. A. & Todi, S. V. 2015 The deubiquitinase ataxin-3 requires Rad23 and DnaJ-1 for its neuroprotective role in Drosophila melanogaster. *Neurobiology of disease* **82**, 12-21. (DOI:http://dx.doi.org/10.1016/j.nbd.2015.05.010).

[129] Howarth, J. L., Kelly, S., Keasey, M. P., Glover, C., Lee, Y. B., Mitrophanous, K., Chapple, J. P., Gallo, J. M., Cheetham, M. E. & Uney, J. B. 2007 Hsp40 Molecules That Target to the Ubiquitin-proteasome System Decrease Inclusion Formation in Models of Polyglutamine Disease. *Molecular therapy : the journal of the American Society of Gene Therapy* **15**, 1100-1105. (DOI:10.1038/sj.mt.6300163).

[130] Labbadia, J., Novoselov, S. S., Bett, J. S., Weiss, A., Paganetti, P., Bates, G. P. & Cheetham, M. E. 2012 Suppression of protein aggregation by chaperone modification of high molecular weight complexes. *Brain* **135**, 1180-1196. (DOI:10.1093/brain/aws022).

[131] Kakkar, V., Mansson, C., de Mattos, E. P., Bergink, S., van der Zwaag, M., van Waarde, M. A., Kloosterhuis, N. J., Melki, R., van Cruchten, R. T., Al-Karadaghi, S., et al. 2016 The S/T-Rich Motif in the DNAJB6 Chaperone Delays Polyglutamine Aggregation and the Onset of Disease in a Mouse Model. *Mol Cell*. (DOI:10.1016/j.molcel.2016.03.017).

[132] Ottaviani, D., Marin, O., Arrigoni, G., Franchin, C., Vilardell, J., Sandre, M., Li, W., Parfitt, D. A., Pinna, L. A., Cheetham, M. E., et al. 2016 Protein kinase CK2 modulates HSJ1 function through phosphorylation of the UIM2 domain. *Hum Mol Genet*. (DOI:10.1093/hmg/ddw420).

[133] Wang, B., Abraham, N., Gao, G. & Yang, Q. 2016 Dysregulation of autophagy and mitochondrial function in Parkinson's disease. *Transl Neurodegener* **5**, 19. (DOI:10.1186/s40035-016-0065-1).

[134] Kitada, T., Asakawa, S., Hattori, N., Matsumine, H., Yamamura, Y., Minoshima, S., Yokochi, M., Mizuno, Y. & Shimizu, N. 1998 Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. *Nature* **392**, 605-608. (DOI:10.1038/33416).

[135] Pemberton, S., Madiona, K., Pieri, L., Kabani, M., Bousset, L. & Melki, R. 2011 Hsc70 protein interaction with soluble and fibrillar alpha-synuclein. *J Biol Chem* **286**, 34690-34699. (DOI:10.1074/jbc.M111.261321).

[136] McLean, P. J., Kawamata, H., Shariff, S., Hewett, J., Sharma, N., Ueda, K., Breakefield, X. O. & Hyman, B. T. 2002 TorsinA and heat shock proteins act as molecular chaperones: suppression of alpha-synuclein aggregation. *J Neurochem* **83**, 846-854.

[137] Auluck, P. K., Chan, H. Y., Trojanowski, J. Q., Lee, V. M. & Bonini, N. M. 2002 Chaperone suppression of alpha-synuclein toxicity in a Drosophila model for Parkinson's disease. *Science* **295**, 865-868. (DOI:10.1126/science.1067389).

[138] Durrenberger, P. F., Filiou, M. D., Moran, L. B., Michael, G. J., Novoselov, S., Cheetham, M. E., Clark, P., Pearce, R. K. & Graeber, M. B. 2009 DnaJB6 is present in the core of Lewy bodies and is highly up-regulated in parkinsonian astrocytes. *Journal of neuroscience research* **87**, 238-245. (DOI:10.1002/jnr.21819).

[139] Shorter, J. 2011 The Mammalian Disaggregase Machinery: Hsp110 Synergizes with Hsp70 and Hsp40 to Catalyze Protein Disaggregation and Reactivation in a Cell-Free System. *PLoS ONE* **6**, e26319. (DOI:10.1371/journal.pone.0026319).

[140] Munoz-Lobato, F., Rodriguez-Palero, M. J., Naranjo-Galindo, F. J., Shephard, F., Gaffney, C. J., Szewczyk, N. J., Hamamichi, S., Caldwell, K. A., Caldwell, G. A., Link, C. D., et al. 2014 Protective role of DNJ-27/ERdj5 in Caenorhabditis elegans

models of human neurodegenerative diseases. *Antioxidants & redox signaling* **20**, 217-235. (DOI:10.1089/ars.2012.5051).

[141] Dawson, T. M. & Dawson, V. L. 2010 The role of parkin in familial and sporadic Parkinson's disease. *Mov Disord* **25 Suppl 1**, S32-39. (DOI:10.1002/mds.22798).

[142] Rose, J. M., Novoselov, S. S., Robinson, P. A. & Cheetham, M. E. 2011 Molecular chaperone-mediated rescue of mitophagy by a Parkin RING1 domain mutant. *Hum Mol Genet* **20**, 16-27. (DOI:10.1093/hmg/ddq428).

[143] Kakkar, V., Kuiper, E. F., Pandey, A., Braakman, I. & Kampinga, H. H. 2016 Versatile members of the DNAJ family show Hsp70 dependent anti-aggregation activity on RING1 mutant parkin C289G. *Sci Rep* **6**, 34830. (DOI:10.1038/srep34830).

[144] Bloom, G. S. 2014 Amyloid-beta and tau: the trigger and bullet in Alzheimer disease pathogenesis. *JAMA Neurol* **71**, 505-508. (DOI:10.1001/jamaneurol.2013.5847).

[145] Tampellini, D., Capetillo-Zarate, E., Dumont, M., Huang, Z., Yu, F., Lin, M. T. & Gouras, G. K. 2010 Effects of synaptic modulation on β-amyloid, synaptophysin and memory performance in Alzheimer's disease transgenic mice. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **30**, 14299-14304. (DOI:10.1523/JNEUROSCI.3383-10.2010).

[146] Evans, C. G., Wisen, S. & Gestwicki, J. E. 2006 Heat shock proteins 70 and 90 inhibit early stages of amyloid beta-(1-42) aggregation in vitro. *J Biol Chem* **281**, 33182-33191. (DOI:10.1074/jbc.M606192200).

[147] Mansson, C., Arosio, P., Hussein, R., Kampinga, H. H., Hashem, R. M., Boelens, W. C., Dobson, C. M., Knowles, T. P., Linse, S. & Emanuelsson, C. 2014 Interaction of the molecular chaperone DNAJB6 with growing amyloid-beta 42 (Abeta42) aggregates leads to sub-stoichiometric inhibition of amyloid formation. *J Biol Chem* **289**, 31066-31076. (DOI:10.1074/jbc.M114.595124).

[148] Abisambra, J. F., Jinwal, U. K., Suntharalingam, A., Arulselvam, K., Brady, S., Cockman, M., Jin, Y., Zhang, B. & Dickey, C. A. 2012 DnaJA1 antagonizes constitutive Hsp70-mediated stabilization of tau. *Journal of molecular biology* **421**, 653-661. (DOI:10.1016/j.jmb.2012.02.003).

[149] Sahara, N., Maeda, S., Yoshiike, Y., Mizoroki, T., Yamashita, S., Murayama, M., Park, J. M., Saito, Y., Murayama, S. & Takashima, A. 2007 Molecular chaperone-mediated tau protein metabolism counteracts the formation of granular tau oligomers in human brain. *Journal of neuroscience research* **85**, 3098-3108. (DOI:10.1002/jnr.21417).

[150] Brehme, M., Voisine, C., Rolland, T., Wachi, S., Soper, J. H., Zhu, Y., Orton, K., Villella, A., Garza, D., Vidal, M., et al. 2014 A Chaperome Sub-Network Safeguards Proteostasis in Aging and Neurodegenerative Disease. *Cell reports* **9**, 1135-1150. (DOI:10.1016/j.celrep.2014.09.042).

[151] Ling, S. C., Polymenidou, M. & Cleveland, D. W. 2013 Converging mechanisms in ALS and FTD: disrupted RNA and protein homeostasis. *Neuron* **79**, 416-438. (DOI:10.1016/j.neuron.2013.07.033).

[152] Novoselov, S. S., Mustill, W. J., Gray, A. L., Dick, J. R., Kanuga, N., Kalmar, B., Greensmith, L. & Cheetham, M. E. 2013 Molecular chaperone mediated late-stage neuroprotection in the SOD1(G93A) mouse model of amyotrophic lateral sclerosis. *PLoS One* **8**, e73944. (DOI:10.1371/journal.pone.0073944).

[153] Shinder, G. A., Lacourse, M.-C., Minotti, S. & Durham, H. D. 2001 Mutant Cu/Zn-Superoxide Dismutase Proteins Have Altered Solubility and Interact with Heat Shock/Stress Proteins in Models of Amyotrophic Lateral Sclerosis. *Journal of Biological Chemistry* **276**, 12791-12796. (DOI:10.1074/jbc.M010759200).

[154] Takeuchi, H., Kobayashi, Y., Yoshihara, T., Niwa, J., Doyu, M., Ohtsuka, K. & Sobue, G. 2002 Hsp70 and Hsp40 improve neurite outgrowth and suppress intracytoplasmic aggregate formation in cultured neuronal cells expressing mutant SOD1. *Brain research* **949**, 11-22.

[155] Chen, H. J., Mitchell, J. C., Novoselov, S., Miller, J., Nishimura, A. L., Scotter, E. L., Vance, C. A., Cheetham, M. E. & Shaw, C. E. 2016 The heat shock response plays an important role in TDP-43 clearance: evidence for dysfunction in amyotrophic lateral sclerosis. *Brain* **139**, 1417-1432. (DOI:10.1093/brain/aww028).