

## Review



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DNAJ Proteins in neurodegeneration:  
essential and protective factors

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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 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 also discuss the role of DNAJ proteins as direct causes of inherited neurodegeneration via mutations in *DNAJ* family genes.

This article is part of the theme issue 'Heat shock proteins as modulators and therapeutic targets of chronic disease: an integrated perspective'.

## 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 characterized by the accumulation of extracellular amyloid- $\beta$  plaques and intracellular tangles of phosphorylated tau; Parkinson's disease (PD) is associated with intracellular deposits of  $\alpha$ -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 ageing [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 endpoint 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 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 the conformation, concentration and location that are 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 compartmentalization, 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  $\alpha$ -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].

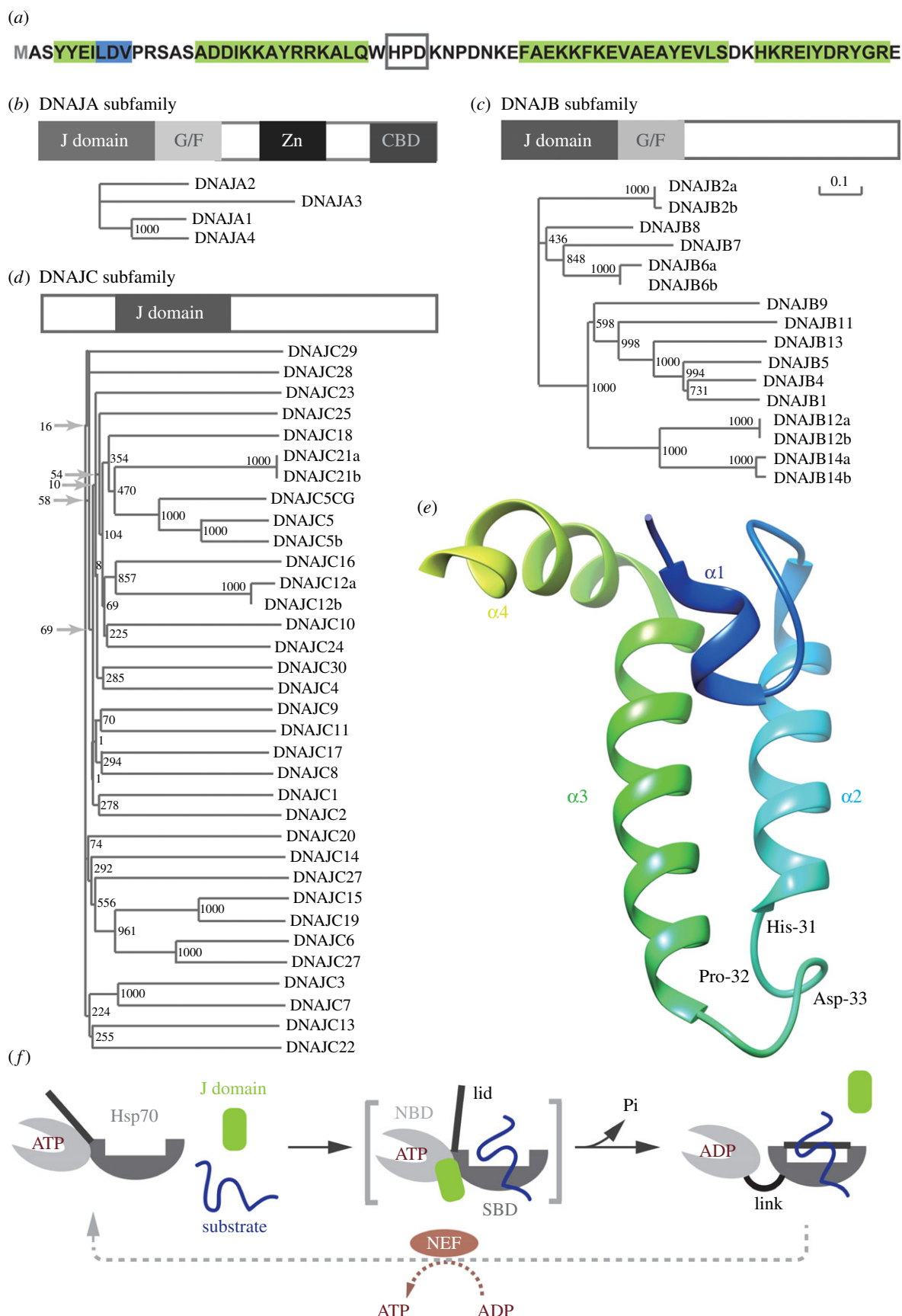
DNAJ protein family members can be divided into three subtypes depending on their domain composition (class I, II or III, also called A, B or C; [16]) (figure 1). Class I (DNAJA) DNAJ proteins are the most similar to the eponymous *E. coli* DnaJ protein and contain the canonical domain structure of an 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, tetratricopeptide 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 [60]. However, mutations in some DNAJ proteins cause non-neurodegenerative disorders; for example, mutations in *DNAJB13* cause primary ciliary dyskinesia [35], mutations in *DNAJC12* cause hyperphenylalanemia [45] and mutations in *DNAJC21* cause bone marrow failure syndrome [53]. 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) [61]. As a type II DNAJ



**Figure 1.** The J domain and DNAJ subfamilies. (a) The amino acid sequence of the DNAJB2 J domain with  $\alpha$ -helices (green),  $\beta$ -sheet (blue) and histidine–proline–aspartic acid (HPD) motif (boxed) highlighted. (b–d) Phylogenetic trees of DNAJ subfamilies and schematic illustrations of their conserved domains. Protein sequence alignments were performed using a Blossum scoring matrix in ClustalX. Bootstrap value is presented at right corner in (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  $\alpha$ -helices 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 left-substrate binding. Nucleotide exchange factors (NEF) (brown) complete the cycle by stimulating the exchange of ADP for ATP and substrate release. (Online version in colour.)

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

DNAJ gene (AKA)	mutation/result	disease	inheritance	references
DNAJB1 (HDJ1/HSP40)	400 kb deletion on chromosome 19 resulting in N-terminal DNAJB1 chimeric in-frame fusion with PKA catalytic domain	fibrolamellar hepatocellular carcinoma	somatic	[20]
DNAJB2 (HSJ1)	c.352+1G>A resulting in intron 5 retention c.229+1G>A resulting in intron 4 retention c.14A>G, p.Y5C (J-domain mutation) c.619-1G>A resulting in splice site deletion c.309delC, p.F103fsX 3.8 kb deletion resulting in J-domain deletion	distal hereditary motor neuropathy  Charcot Marie Tooth disease type 2  spinal muscular atrophy/juvenile Parkinsonism	recessive	[21–25]
DNAJB5 (HSC40)	c.43C>T, p.P15S (J-domain mutation)	hereditary myoclonus and progressive distal muscular atrophy	recessive	[25]
DNAJB6 (MRJ)	c.265T>A, p.F89I c.271T>A, p.F91I c.271T>C, p.F91L c.273C>G, p.F93I 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	limb-girdle muscular dystrophy	dominant	[26,27–34]
DNAJB13	c.833T>G, p.M278R c.68+1G>C, p.Y24X	primary ciliary dyskinesia type 34	recessive	[35]
DNAJC3 (p58)	c.508C>T, p.R194X 72 kb deletion resulting in loss of exons 6–12	combined cerebellar and peripheral ataxia with hearing loss and diabetes mellitus	recessive	[36]
DNAJC5 (CSP $\alpha$ )	c.346_348delCTC, p.L116 $\Delta$ c.344T>G, p.L115R	adult-onset neuronal ceroid lipofuscinosis	dominant	[37,38]
DNAJC6 (auxilin)	c.801-2A>G c.2371C>T, p.G791X 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 c.2223A>T, p.T741X c.2517del, p.F389LfsX22 c.2779A>G, p.R927G (J-domain mutation) 80 kb deletion of exons 5–19	autosomal recessive juvenile Parkinsonism early-onset Parkinson's disease              early-onset obesity, mental retardation and epilepsy	recessive	[39–42,43]

(Continued.)

Table 1. (Continued.)

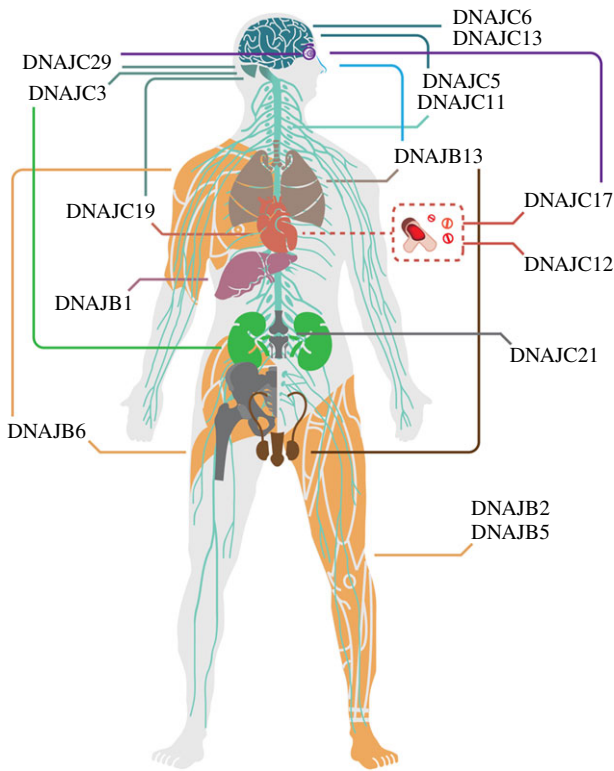
DNAJ gene (AKA)	mutation/result	disease	inheritance	references
Dnajc11	c.1524+56T>A (mice only) resulting in cryptic splicing, p.K508fsX43	spasticity, MN pathology	recessive	[44]
DNAJC12 (JDP1)	c.298-968_503-2603del resulting in exon 4 deletion c.215G>C, p.R72P c.158-2A>T resulting in intron 3 splice site mutation	hyperphenylalaninemia, mild, non-BH4 deficient	recessive	[45]
DNAJC13 (RME-8)	c.2564A>G, p.N855R	autosomal dominant Parkinson's disease	dominant	[46]
DNAJC17	c.681G>A (r.601_681del), p.Y201_A227del	retinitis pigmentosa and hypogammaglobulinemia	recessive	[47]
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	[48–52]
DNAJC21	c.517C>T, p.R173X c.983+1G>T, p.G299AfsX2 c.94C>G, p.P32A c.793G>T, p.Q265X	bone marrow failure syndrome type 3	recessive	[53]
DNAJC29 (sacin)	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	[54–59]

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 UIMs, which can bind ubiquitylated client proteins and target them to the proteasome for degradation [62]. 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 [63]. 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 [64,21]. 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 [65,66]. 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 [22]. There have been additional reports of patients with this mutation recently [67]. 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 [23]. 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 [21]. 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) [24]. 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; [25]).

### (b) DNAJB5 (HSC40)

DNAJB5 was originally identified as being similar to DNAJB1 [68] and has since been shown to interact with HSP70 [69]. 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 [25].



**Figure 2.** Pathogenic consequences of DNAJ family mutations. Schematic illustration of a human body demonstrating 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). (Online version in colour.)

Morpholino-mediated knockdown of DNAJB5 in zebrafish revealed abnormalities in peripheral nerve axon structure, but no effect on muscle architecture [25]

### (c) DNAJB6 (MRJ)

DNAJB6 is a ubiquitous protein with high expression levels in the brain and detectable protein in muscle [70]. Alternative splicing of the DNAJB6 gene produces two isoforms: a 36 kDa nuclear isoform and a 26 kDa cell stress-responsive cytosolic form [71]. 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 [72]. 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 [73]. Patients with *DNAJB6* mutations have myofibrillar aggregates containing ubiquitin, TDP-43 and p62, suggesting defective protein clearance [26,74], which are also observed in *Dnajb6* F93L transgenic mice [75]. *Drosophila* mutants recapitulating patient mutations result in loss of DNAJB6-dependent anti-aggregation activity [76].

### (d) DNAJC3 (p58)

DNAJC3 is a 58 kDa DNAJ protein that is targeted to the cytoplasmic face of the ER [77,78]. DNAJC3 can also bind and inhibit the UPR sensor PERK in the ER, suggesting a role in regulating the UPR [79,80]. DNAJC3 can recruit cytosolic HSP70 to the face of the ER and work with Sec61 as part of the translocation machinery [81]. Knockdown of DNAJC3 results in accumulation of misfolded protein in the ER and activation of the UPR [82] and *Dnajc3* knockout mice have decreased ability to cope with ER stress [81,83]. Mutations in DNAJC3 cause multisystemic neurodegeneration, including early-onset cerebellar ataxia and peripheral neuropathy, alongside diabetes mellitus [36]. Interestingly, *Dnajc3* knockout mice also show a diabetic phenotype [83] 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 [84].

### (e) DNAJC5 (CSP $\alpha$ )

DNAJC5 is a secretory vesicle protein found in both neuronal and non-neuronal tissues; however, the main  $\alpha$ -isoform is only expressed in the brain [85]. DNAJC5 is characterized by a cysteine-rich region and is targeted to post-Golgi membranes via palmitoylation [86]. DNAJC5 has a role in binding and folding many proteins required at the synapse, such as SNAP-25, syntaxins and synaptotagmins [87–89], where it acts as a chaperone with the constitutive HSP70, HSC70 (HSPA8) [90]. DNAJC5, therefore, most likely plays a key role at the synapse as a chaperone [91,92]. Mutations in *DNAJC5* cause autosomal dominant adult onset neuronal ceroid lipofuscinosis (ANCL), an accumulation of autofluorescent lysosomal waste (known as lipofuscin) that causes a progressive neurodegenerative disorder characterized by ataxia, seizures and dementia [93]. 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 [37,38,94,95]. The location of the mutations in the cysteine-rich region suggests a defect in the membrane trafficking of patient DNAJC5 and subsequent protein aggregation [96,97]. DNAJC5 interacts with another ANCL disease-causing protein, palmitoyl-protein thioesterase 1 (PPT1). PPT1 is accumulated in DNAJC5 patient brains and has decreased activity, suggesting a link between ANCL and palmitoylation of synaptic proteins [98]. *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 [85]. Interestingly, DNAJC5 mutations in *Caenorhabditis elegans* lead to sensory neuron dysfunction that could be rescued by treatment with resveratrol [99].

### (f) DNAJC6 (auxilin)

Another well-characterized vesicle-associated protein is DNAJC6, which has a role in uncoating clathrin-coated vesicles [100]. DNAJC6 binds clathrin via its C-terminal clathrin binding-domain [101,102]. The clathrin-coating and uncoating cycle is well characterized; clathrin triskelions form coated pits at the pre-synaptic membrane around the intended cargo. Before fusing with the endosome, the vesicles need to be uncoated by HSC70, following recruitment and activation by DNAJC6 [103,104]. In neurons, this process is

vital for synaptic vesicle recycling. Mutations in *DNAJC6* were first associated with autosomal recessive juvenile parkinsonism (ARJP) [39,40]. 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 [41]. A recent study also identified variants in *DNAJC6* that are associated with early-onset PD, which has a later onset than ARJP [42]. 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 [105,106]. DNAJC11 was later identified as a mitochondrial protein [107], 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 [108]. 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 [44]. 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 [44].

### (h) DNAJC13 (RME-8)

DNAJC13 is an endocytic protein that has been shown to localize to early and recycling endosomes [109]. 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 [109]. DNAJC13 interacts with the retromer complex [110] 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 [46]; 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 [111]. 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 [112]. Further analysis revealed that other DNAJC13 variants (E1740Q, R1615H, L2120W) might be associated with increased risk of PD [113,114].

### (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 [115]. 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 [48]. Recently, single nucleotide deletions and splice deletions have also been identified with associated disease features [49,50,51].

### (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 [116,117]. 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 [118]. 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 [119]. 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 [54], although more than 150 other patient mutations are now known worldwide, including large-scale deletions [120,121]. 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 in an *E. coli* DnaJ and CbpA temperature-sensitive mutant, and interestingly there are two patient missense mutations located in the J-domain (R4331Q and E4343K) [55,56]. *Dnajc29* knockout mice have ataxic symptoms with peripheral neuropathy and progressive Purkinje cell loss, recapitulating the human disorder [122]. Furthermore, *Dnajc29* null mice motor neurons have elongated mitochondria and accumulations of neurofilaments [122]. DNAJC29 interacts with the mitochondrial fission protein DRP1 [118] and recent work using patient fibroblasts has shown that there is a reduction of DRP1 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 [123].

## 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 ageing. 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, TATA box-binding protein (TBP)) (figure 3). Ubiquitylated inclusions of aggregated protein are characteristic of these diseases [124].

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) [125]. In cells overexpressing polyQ-expanded ataxin-1, knockdown of DNAJC29 has been shown to enhance ataxin-1-mediated toxicity, indicating a protective role against polyQ-expanded ataxin-1 toxicity [116]. 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 proteasomal degradation through J-domain, or diminish this process by preserving ubiquitylated Atx3 via the UIM domain [126]. 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 [127]. 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 [128,129]. DNAJB1 has been also shown to increase solubility of polyQ-expanded AR and also enhance proteasome-mediated degradation in cells, an effect again amplified in the presence of HSP70 [130,131].






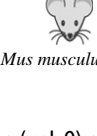
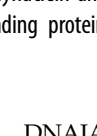
A screen of several different DNAJA and DNAJB proteins revealed that a subfamily of DNAJB proteins were the most efficient at reducing polyQ aggregation [69,132]. This subfamily includes DNAJB2a, DNAJB6b and DNAJB8, which are closely related (figure 1c), but the effect is also dependent on their sub-cellular localization. *In vitro* studies with purified proteins have shown that DNAJB6b can suppress the formation of amyloid-like fibrils of polyQ peptides [133]. Moreover, DNAJB6b and DNAJB8 were shown to suppress polyQ aggregation and related toxicity in cells and transgenic *Xenopus laevis* models [132]. Genome-wide RNA interference screen on transgenic *C. elegans* expressing polyQ proteins identified DNAJ as a suppressor of polyQ aggregation [134]. Interestingly, DNAJB6b and DNAJB8 are effective in suppressing the aggregation not only of polyQ-expanded htt, but also of other disease-related polyQ-expanded proteins, such as Atx3 and the androgen receptor (SBMA) [132]. 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 [135]. 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 [62,136]. 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 [136]. *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 [137].

*In vivo* investigation in *Drosophila* models for HD identified dHDJ1, the homologue of DNAJB1, as a suppressor of polyQ-driven toxicity [138]. A separate study showed that the DNAJB1-induced reduction of eye degeneration in transgenic polyQ *Drosophila* was enhanced by *Drosophila* HSC70cb and its human homologue APG-1, while DNAJB1 also had an effect in the absence of HSP70 [139]. Moreover, dMRJ, the *Drosophila* orthologue of the human DNAJB6, was recruited in the polyQ inclusions and was shown to suppress polyQ-mediated toxicity in flies [140]. 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 [140]. Expression of dHDJ1 on mutant Atx3-expressing flies restored eye structure, an effect attributed to both J- and C-terminal domains. Interestingly, the effect of dHDJ1 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, which 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 [141]. 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 [142]. 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 [143].

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 [144]. 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 [145,146]. 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 [145]. 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 [145].



chaperone	effect	chaperone	effect
 <i>in vitro</i>	DNAJB1 +Hsp70	↓ polyQ amyloid-like fibril formation	↓ Aβ aggregation
	DNAJB1	↓ tau aggregation	↓ α-synuclein, Aβ fibril formation
		↓ Aβ amyloid fibril formation	
 cell models	DNAJB6b	↓ htt amyloid-like fibril formation	
	DNAJA1	↓ Atx1, α-synuclein aggregation	↓ α-synuclein aggregation
		↑ tau degradation	
	DNAJB1	↓ htt, α-synuclein inclusions/aggregates	
	DNAJB1 (+Hsp70)	↓ htt-induced cytotoxicity	↑ proliferation (htt)
		↑ AR solubility and degradation	↑ Parkin, SOD1 aggregation
		↑ neurite outgrowth in SOD1	
	DNAJB2	↓ htt, Parkin, SOD1, TDP-43 aggregation	↑ Parkin (C289G) induced mitophagy
	↓ Parkin misfolding	↑ TDP-43 folding	
	DNAJB6b	↓ polyQ, Atx3, AR, Parkin, Aβ aggregation	
	DNAJB8	↓ polyQ and Parkin aggregation	
 <i>Caenorhabditis elegans</i>	DNAJB2b	↑ neuronal function in htt	↓ neuronal death from htt
	DNAJC10	↓ α-synuclein aggregation and toxicity	↓ Aβ toxicity
 <i>Drosophila melanogaster</i>	dHDJ1	↓ htt toxicity and eye degeneration	↑ polyQ aggregation
		↑ soluble polyQ	
 <i>Drosophila melanogaster</i>	dHDJ1 +Hsp70	↓ toxicity of mutAtx3	
	DNAJB1 (+Hsp70)	↓ eye degeneration in polyQ	
 <i>Mus musculus</i>	dMRJ	↓ polyQ toxicity	↑ soluble polyQ
	DNAJ-1	↓ eye degeneration (SCA6 models)	
 <i>Mus musculus</i>	DNAJB2a	↓ htt aggregation, inclusion formation and size	↑ neurological performance (modest)
	DNAJB2a	↑ muscle function	↑ motor neuron survival
		↑ weight	
	DNAJB6	↓ htt inclusion formation	↑ neurological performance
		↑ lifespan	

**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.  $\alpha$ -synuclein and parkin implicated in Parkinson's disease and amyloid- $\beta$  (A $\beta$ ) 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. (Online version in colour.)

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 lifespan [146]. *In vitro* studies suggest this was through an effect on primary nucleation of polyQ aggregation [146]. 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 [147]. 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) $\alpha$ -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,  $\alpha$ -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 [148]. Furthermore, mutations in SNCA, which encodes  $\alpha$ -synuclein, have been associated with PD and mutations in PARK2, which encodes Parkin, lead to the autosomal recessive juvenile form of the disease ARJPD [149].

DNAJB1 has been shown to slow down the assembly of  $\alpha$ -synuclein fibrils and increase the binding of HSC70 to fibrillar  $\alpha$ -synuclein *in vitro* [150]. In addition, DNAJA1 was reported to bind  $\alpha$ -synuclein fibrils and increase binding of HSC70 to preformed fibrils *in vitro*; however, DNAJA1 alone had no effect on the assembly of  $\alpha$ -synuclein fibrils [150]. Both

DNAJA1 and DNAJB1 have been shown to co-localize with  $\alpha$ -synuclein inclusions in cells [151]. Post-mortem PD brain tissues 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 [152,153]. Moreover, co-expression of  $\alpha$ -synuclein and either DNAJA1 or DNAJB1 dramatically decreased  $\alpha$ -synuclein aggregates in cells [151]. Finally, DNAJB1 combined with HSP70 and HSP110 can recover amorphous  $\alpha$ -synuclein aggregates, while they also enhance the effect of non-mammalian Hsp104 to remodel  $\alpha$ -synuclein amyloids *in vitro* [154]. *In vivo* overexpression of the human homologue DNAJC10 in *C. elegans* decreased  $\alpha$ -synuclein aggregates and toxicity [155].

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 play an important role in mitochondria dynamics and function [156]. 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 [157]. In contrast to polyQ, most cytosolic DNAJ proteins tested could reduce Parkin RING1 domain mutant (C289G) aggregation, and for DNAJB6 and DNAJB8 this was less reliant on their S/T region and more dependent on HSP70 [158]. This illustrates that chaperone manipulation can be versatile and unique to individual protein clients.

### (c) Tau and amyloid- $\beta$

Extracellular amyloid plaques composed of amyloid- $\beta$  (A $\beta$ ) peptides and intraneuronal tau neurofibrillary tangles form the characteristic pathophysiological profile of Alzheimer's

disease (AD) [159]. Although accumulation of A $\beta$  fibrils occurs extracellularly on senile plaques, intraneuronal generation of A $\beta$  has been correlated to synapse damage and enhanced intracellular accumulation in AD-transgenic mice [160].

Interestingly, DNAJB1 has been shown to enhance the effect of HSP70 *in vitro* in reducing A $\beta$  aggregation through targeting smaller species such as oligomers [161]. Moreover, DNAJB6, and specifically the DNAJB6b isoform, which is localized in both the nucleus and the cytosol, has been shown to be a potent suppressor of A $\beta$ 42 aggregation *in vitro* preventing the formation of amyloid fibrils by interacting with the early formed aggregates during nucleation [162]. In a cellular model of AD overexpressing GFP-tagged A $\beta$ 42, DNAJB6 was shown to reduce intracellular A $\beta$  aggregation and required interaction with HSP70. In *C. elegans* models of A $\beta$ , overexpression of DNAJ27 (orthologue of mammalian DNAJC10) had a protective role against A $\beta$ -induced toxicity; however, overexpression of human DNAJC10 in A $\beta$  worms had no effect [155].

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 stabilized tau and halted degradation [163]. 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* [164]. Finally, Brehme *et al.* have shown that knockdown of DNAJA1 and DNAJA4 or the *C. elegans* homologues can increase the aggregation and toxicity of A $\beta$ 42 [165].

#### (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 predominantly 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 [166].

Both DNAJB2 isoforms have been shown to significantly reduce mutant SOD aggregation in an overexpression cell model [22,167]. *In vivo* investigation of DNAJB2a overexpression in double transgenic SOD1<sup>G93A</sup> 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 [167].

DNAJB1 co-immunopurified with mutant SOD1, but not with wild type or endogenous SOD1 from cell extracts [168]. 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) [169]. Finally, Chen *et al.* [170] 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 [170].

## 5. Conclusion

The essential role of molecular chaperones in maintaining neuronal proteostasis is highlighted by the 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.

**Data accessibility.** This article has no additional data.

**Competing interests.** We declare we have no competing interests.

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