CHAPTER 2

Cellular Protein Quality Control and the Evolution of Aggregates in SCA3

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Abstract

Aims: A characteristic of polyglutamine (polyQ) diseases is the increased propensity of disease proteins to aggregate, which is thought to be a major contributing factor to the underlying neurodegeneration. Healthy cells contain mechanisms for handling protein damage, the protein quality control (PQC), which must be impaired or inefficient to permit proteotoxicity under pathological conditions.

Methods: We used a quantitative analysis of immunohistochemical stainings of the pons of 8 patients with the polyQ disorder SCA3. We employed the anti-polyglutamine antibody 1C2, antibodies against p62 that is involved in delivering ubiquitinated protein aggregates to autophagosomes, antibodies against the chaperones HSPA1A and DNAJB1 and the proteasomal stress marker UBB+1.

Results: The 1C2 antibody stained Neuronal Nuclear Inclusions (NNI), Diffuse Nuclear Staining (DNS), Granular Cytoplasmic Staining (GCS) and combinations, with reproducible distribution. P62 always colocalized with 1C2 in NNI. DNS and GCS co-stained with a lower frequency. UBB+1 was present in a subset of neurons with NNI. A subset of UBB+1 containing neurons displayed increased levels of HSPA1A, while DNAJB1 was sequestered into the NNI.

Conclusion: Based on our results, we propose a model for the aggregation associated pathology of SCA3: GCS and DNS aggregation likely represents early stages of pathology, which progresses towards formation of p62 positive NNI. A fraction of NNI exhibits UBB+1 staining, implying proteasomal overload at a later stage. Subsequently, the stress inducible HSPA1A is elevated while DNAJB1 is recruited into NNIs. This indicates that the stress response is only induced late when all endogenous PQC systems have failed.
Introduction

Protein aggregation can be caused by chemical, oxidative and heat stress as well as by protein misfolding or mutations.¹ It is also a characteristic feature of many neurodegenerative diseases, including the polyglutamine (polyQ) diseases.² Here, an expansion of a poly-CAG repeat sequence within a gene coding for a disease specific protein causes the expansion of a polyglutamine sequence resulting in aggregation of the disease protein, which is considered to be a major contributing factor to the severe neurodegeneration present in these diseases.¹⁻⁴

Spinocerebellar ataxia type 3 (SCA3), a polyQ disease, is the most frequent autosomal dominant spinocerebellar ataxia.⁵ In healthy individuals the causative ATXN3 gene, situated on chromosome 14q32.1 contains 12-40 CAG-repeats, while in patients it encompasses 61-84 CAG-repeats.³ The best-described protein aggregates in SCA3 are neuronal nuclear inclusions (NNI), which have been demonstrated to sequester numerous other proteins (e.g. transcription factors, heat shock proteins).¹ However, the relation between the NNI and neuronal death remains elusive. While an animal model demonstrates that ataxin-3 toxicity depends on nuclear localization,⁶ nuclear aggregates are also present in areas unaffected by neurodegeneration in SCA3 patients (e.g. medial thalamic nuclei).⁶,⁷ Other types of aggregates exist, but received less attention.⁸,⁹

To deal with misfolded and aggregating protein, cells contain several biological pathways, referred to as the protein quality control (PQC) system, which include refolding (e.g. HSP70 chaperones) and degradation pathways (e.g. ubiquitin-proteasome system, autophagy). The evolutionary highly conserved HSP70 chaperones act together with the DNAJ family of co-chaperones in multiple folding processes.¹⁰ The human HSPA1A is a strongly stress inducible HSP70 member and an excellent marker of proteotoxic stress and, if present at elevated levels, can protect against acute proteotoxic stress by assisting protein refolding.¹² However HSPA1A is not very effective in responding to and protecting against chronic stress, like expression of polyQ disease protein.¹³ For the latter, up-regulation of DNAJ proteins seems to be more effective.¹³ In the present study, we focused on DNAJB1, as it has been reported to be included into polyQ inclusions in post mortem tissue⁴ and expression levels in fibroblast cultures of SCA3 patients were associated with the age of onset of these patients.¹⁵ Also, DNAJB1 was found to reduce polyQ aggregation in a HSPA1A
dependent manner in a cell model,\textsuperscript{13,16} by assisting proteasomal degradation of polyQ proteins.\textsuperscript{17}

The ubiquitin proteasome system (UPS) degrades target proteins into oligopeptides, thereby eliminating unnecessary or misfolded proteins.\textsuperscript{18} Target proteins tagged with a specific polyubiquitin flag are transferred to the proteasome via shuttle proteins. In polyQ diseases like SCA3, the aggregates are ubiquitinated, indicating that the disease protein may be flagged for UPS degradation.\textsuperscript{19} However UPS degradation of aggregated protein is either inefficient or impossible. The presence of ubiquitinated aggregates therefore likely represents the overloading or failure of the proteasome to degrade soluble polyQ protein.\textsuperscript{20} Ubiquitinated proteins can also be recognized by e.g. the p62 protein to assemble them into aggregates and shuttle them to autophagic vesicles.\textsuperscript{21–24}

Although it is debated how and at what stage in disease, general proteasomal overload does seem to occur during polyQ disease progression. If blocked by aggregated protein material, the UPS also becomes less competent at degrading normal, unaggregated target protein. This may be deduced from the increased presence of the ubiquitin transcription frameshift mutant UBB\textsuperscript{+1} in post-mortem tissue of SCA3 patients. Molecular misreading of ubiquitin to UBB\textsuperscript{+1} also occurs in healthy cells, where it is quickly degraded by a normally functioning UPS and thus hardly detectable.\textsuperscript{25,26}

In the present study, we performed a quantitative analysis of aggregation profiles in the pontine nuclei of 8 SCA3 patients in relation to changes in components of the PQC. We found a reproducible distribution of the different neuronal aggregate types and observed UBB\textsuperscript{+1} and aberrant HSPA1A/DNAJB1 expression in a specific co-staining pattern related to aggregate formation, from which we propose a model for neuronal cell pathology and PQC failure in SCA3.

Results

Characterization of polyQ aggregates in the pontine nuclei of SCA3 patients

Using the 1C2 antibody, which recognizes any polyQ repeat of 38+,\textsuperscript{27} we first analyzed the types of aggregates present in the pontine nuclei of 8 SCA3 brains (Fig 1). Individual neurons exhibited a variety of staining patterns consistent with
Cellular PQC and the evolution of aggregates in SCA3

earlier observations.\(^8\) NNI, defined as clearly demarked and strongly stained round objects within the neuronal nucleus, not in contact with the nuclear membrane, are frequently reported inclusions (Fig 1A). Besides NNI, Diffuse Nuclear Staining (DNS), defined as uncondensed and evenly distributed or slightly grainy (Fig 1B), was detected in a majority neurons. Furthermore, a subset of neurons was found to contain Granular Cytoplasmic Staining (GCS), with the granules being usually smaller than NNIs (Fig 1C).

For each patient, the frequency of the aggregate types was scored by 3 independent observers in 100 neurons. Scoring was highly reproducible (Supplemental Fig 1) and all patients show similar aggregation patterns (Fig 1M), with most neurons containing a mixture of NNI, DNS or GCS, and a notable absence of the NNI/GCS combination (Fig 1M). In this study, the average percentage of neurons showing staining patterns which include NNI was 62% in the fluorescent stainings and 53% in positive contrast.

Another marker frequently used for protein aggregation is the p62 protein [21,22]. The adaptor protein p62 is required for the formation of ubiquitinated protein aggregates in vitro and formation of inclusion bodies in cells; it also binds directly to the autophagosomal marker LC3.\(^{23}\) The tripartite interaction between p62, ubiquitinated protein (aggregates) and LC3 is presumed to mediate delivery of these aggregates to the autophagy system to facilitate aggregate degradation.\(^{21-24}\) The p62 antibody stains NNIs, DNS as well as GCS (Fig 1D-F) but preferentially detects NNI (Fig 1N and Supplemental Fig 1C). In contrast, an ubiquitin antibody decorated all types of aggregates with no preference (Fig 1G-I and Supplemental Fig 1B).

**PolyQ aggregates and protein quality control**

UBB\(^+1\) is a frame shift mutant of ubiquitin, resulting from molecular misreading which occurs at low levels in healthy conditions, and is usually undetectable due to its rapid proteasomal degradation.\(^{25,26}\) Upon proteasomal inhibition, UBB\(^+1\) levels can increase to detectable levels and thus can be used as a suitable marker for proteasomal dysfunction in post-mortem tissue,\(^{28}\) and can by itself exert a toxic influence onto the affected neurons.\(^{29}\) Using double staining with either 1C2 or p62 together with UBB\(^+1\) specific antibodies, we observed that UBB\(^+1\) was levels can increase to detectable levels and thus can be used as a suitable marker for proteasomal dysfunction in post-mortem tissue,\(^{28}\) and can by itself exert a
toxic influence onto the found exclusively in neurons containing NNI (Fig 2) but not DNS or GCS, and located primarily in the NNI. All UBB\textsuperscript{+1} positive NNI co-affected neurons.\textsuperscript{29}

Figure 1. Analysis of aggregate types and frequencies. Immunostaining of polyglutamine aggregates with 1C2 (A-C), p62 (D-F) and ubiquitin (G-I) and 1C2/p62 double staining (J-L). NNIs (arrows) (A,D,F,G), DNS (open arrows) (B,E,F,H) and GCS (arrowheads) (C,F,I) exhibit different staining qualities and intensities, depending on the antibody employed. Note the combination of staining patterns in figure F. In all cases, the 1C2/p62 staining co-localizes in NNI (J-L: 1C2 (green) and p62 (red)). Frequency distribution of 1C2- (M) and p62-positive (N) aggregate types (mean ± 1 SD). (A-C: 1C2 staining with hematoxylen counterstaining; D-F: p62 staining with hematoxylen counterstaining; G-I: ubiquitin staining with hematoxylen counterstaining; J-L: 1C2 – red, UBB\textsuperscript{+1} – green).
Using double staining with either 1C2 or p62 together with UBB\textsuperscript{+1} specific antibodies, we observed that UBB\textsuperscript{+1} was stained with either 1C2 or p62, but only a fraction of 1C2/p62 positive NNI (27\% of NNI containing neurons, 17\% of all neurons, n=300) showed UBB\textsuperscript{+1} labeling, suggesting that only a fraction of neurons containing NNI exhibit signs of proteasomal dysfunction. A central element in protein homeostasis is formed by molecular chaperones, especially those of the family of heat shock proteins (HSPs). Several HSPs have been implicated in disease processes, especially DNAJB1 as a modifier of age of onset in SCA3\textsuperscript{15}. In the pontine nuclei of control cases, all neuronal cells showed moderate DNAJB1 staining primarily in the cytoplasm (Supplemental Fig 2A).

**Figure 2: Co-localization of UBB\textsuperscript{+1} with p62 and 1C2.** Fluorescent double staining with p62/UBB\textsuperscript{+1} (A-C) and 1C2/UBB\textsuperscript{+1} (D-F). UBB\textsuperscript{+1} staining co-localizes with some (arrows) but not all (arrowheads) p62 and 1C2 positive NNI (A-F). (A-C: p62 – red, UBB\textsuperscript{+1} – green; D-F: 1C2 – red, UBB\textsuperscript{+1} – green.)
The same was found in most neuronal cells from the SCA3 patients (Supplemental Fig 2B). Double fluorescent staining of 1C2 and DNAJB1 showed that 85% of all NNI were immunoreactive for DNAJB1 (Supplemental Fig 3A-C). Most of these neurons still showed a diffuse cytoplasmic and nuclear staining (Supplemental Fig 3A-C). However, a small fraction of all neurons (6%) showed a marked decrease of cytoplasmic staining combined with sequestration of the DNAJB1 into the NNI (Supplemental Fig 3D-F). Cells without a NNI never displayed this absence of cytoplasmic DNAJB1 staining.

Finally, we performed double immunolabelling with antibodies against p62, UBB, or DNAJB1 combined with a specific antibody against the stress-inducible member of the HSP70 family, HSPA1A. In most neurons, HSPA1A is found at low basal levels in the cytoplasm both in control and SCA3 brains (Supplemental Fig 2C,D). A majority of NNIs were HSPA1A immunoreactive as revealed by the 1C2 co-staining (Supplemental Fig 4), consistent with in vitro data and previous work in human brains, indicating that HSPA1A is recruited into the NNI. Interestingly, combined staining of UBB with HSPA1A revealed a subset of UBB positive neurons that exhibited a marked up-regulation of HSPA1A (Fig 3A-C).

Double staining of DNAJB1 with HSPA1A revealed that it is precisely cells lacking cytoplasmic DNAJB1 staining and full sequestration of DNAJB1 into the NNI, where HSPA1A intensities were drastically elevated (Fig 3D-F). In fact, we found an inverse correlation between cytoplasmic HSPA1A and DNAJB1 staining intensities (Supplemental Fig 5).

Discussion

In this manuscript, we quantitatively analyzed different forms of protein aggregates in post-mortem brains of SCA3 patients in relation to differential expression of components of the PQC. Furthermore, our data suggest that components of the PQC only respond to and become impaired at a late stage during the aggregation process. From this, we propose a sequence of events taking place during the aggregation process of SCA3. Using 1C2 antibodies as protein aggregation marker, we found neurons not only to contain NNI, the canonical aggregates that hallmark SCA3, but also diffuse aggregates throughout the nucleus (DNS) and granular aggregates in the cytoplasm (GCS), consistent with earlier reports. Frequently, combinations of the 3 staining types
Cellular PQC and the evolution of aggregates in SCA3

were observed, except for the combined CGS/NNI patterns, which were never found. Another aggregation marker, the p62 protein, had a clear preference for NNI, with GCS and DNS type labeling being both less frequent and less intensive.

In parallel to these aggregation patterns, we studied the distribution of PQC markers. As marker for proteasomal overload we used UBB\(^{+1}\).\(^{25,26}\) The presence of UBB\(^{+1}\) in a subset of NNI containing neurons suggests that a fraction of neurons exhibit signs of proteasomal dysfunction and that GCS and DNS may represent earlier less stressful stages of aggregation pathology, since they do not display detectable levels of UBB\(^{+1}\).

Figure 3: DNAJB1 sequestration into NNI and HSPA1A up-regulation. Fluorescent double staining with UBB\(^{+1}\)/HSPA1A (A-C) and DNAJB1/HSPA1A (D-F) showing positivity of UBB\(^{+1}\) in a subset of neurons both with and without changes in DNAJB1 or HSPA1A in neurons. HSPA1A is strongly up-regulated when DNAJB1 is sequestered from the cytosol into the NNIs (arrow), while other neurons retain their staining (arrowhead). (A-C: p62 – red, UBB\(^{+1}\) – green; D-F: HSPA1A – green, DNAJB1– red).
As markers for (proteotoxic) stress, we monitored the expression of the stress inducible HSPA1A chaperone and the co-chaperone DNAJB1. Somewhat surprisingly, HSPA1A was only found to be up-regulated in a subset of UBB positive neurons: in fact, only those UBB positive, NNI containing neurons that concomitantly have lost somatic staining of the HSP70 co-chaperone DNAJB1 showed this HSPA1A up-regulation. This shows that neurons do not display a heat shock response in the presumed early stages of aggregation of the polyQ protein (DNS, GCS).

**Staging of the Aggregation process**
Based on these observations, we propose the following model for the sequence of events in SCA3 neurons (Fig. 4): Neurons containing 1C2-stained GCS (stage 1) and DNS (stage 2) are only rarely positive for p62. Formation of NNI (stage 3) likely represents a more advanced stage of the aggregation process, as they are constitutively labeled by p62.

The progressive protein aggregation next leads to proteasomal impairment (stage 4) as supported by the UBB staining, only present in neurons with NNI. The final stage (stage 5) is characterized by the up-regulation of HSPA1A, while at the same time the co-chaperone DNAJB1 is sequestered into the NNI.

*Figure 4: Model for progressive changes in protein quality control in human SCA3. Aggregating ataxin3 (brown dots) occurs first as GCS, progresses via DNS to NNI. Proteasomal dysfunction, marked by UBB (red dots), only occurs in neurons with NNI. Up-regulation of HSPA1A is associated with DNAJB1 sequestration into NNI. Frequencies of all stages as derived from all quantifications are given in the bars.*
In this model, the transition from cells exhibiting aggregation of disease protein into GCS towards DNS and NNI during stages 1 to 3 is supported by the lack of GCS/NNI combinations and the preferential staining of NNI by the p62 antibody. P62 is implicated in active aggregation processes, as knocking out p62 in model organisms leads to absence of aggregates and enhanced proteotoxicity. This also suggests that NNI are not necessarily toxic and may even reflect active protective mechanisms of temporal storage of aggregating protein. However, while cytoplasmic inclusions might be subject to autophagosomal degradation, this may be less likely for inclusions within the nucleus. While polyQ proteins can be shuttled out of the nucleus, it is a highly aggregation-prone environment in which small aggregates may rapidly nucleate to large aggregates which then can no longer be transported through the nuclear membrane.

Although in vitro data are inconclusive regarding the precise interference of polyQ aggregates with the proteasome, the presence of UBB+ during stage 4 hints at an impairment of the proteasomal system. Its function could either be inhibited due to saturation of the ability to sequester polyQ aggregates (overloading the proteasome) or due to direct sequestration of elements of the proteasome (loss of function).

The up-regulation of HSPA1A demarks the final stage (stage 5) of the progressive aggregation pathology. The constitutive expression of chaperones might be able to temporarily counterbalance the deficit in proteostasis caused by the impairment of proteasomal function. Long term benefits, however seem unlikely. Indeed, the HSP70 overexpressing neurons showed morphological abnormalities (Fig 3) indicative of poor health.

It is striking to note that the stress response seems to be activated only so late during pathogenesis, given its potential to interfere with misfolded proteins. However, the heat shock response (HSR) is self-regulating and induced in a stress-dose dependent manner. Given the chronic nature of the disease and the slow accumulation of misfolded proteins over time, this may just be insufficient to induce the HSR. Only when all other systems fail and misfolded protein or/and aggregates accumulate to high quantities HSR seems activated. At this stage, however, cellular damage may already be too severe and the induction of the HSR, amongst which the elevated expression of HSPA1A, is too late and no longer sufficient to be cytoprotective.
Lastly, it is interesting to note that the HSPA1A induction coincided with a sequestration of the constitutively expressed DNAJB1 in the inclusions. DNAJ proteins are generally found to be good suppressors of polyQ aggregation and toxicity. Moreover, we recently found that differential expression of DNAJB1 in SCA3 patients was correlated to the age at onset in addition to CAG repeat length. This raises the hypothesis that the recruitment of DNAJB1 proteins into the NNI in parallel with its cytosolic disappearance reflects a failure of these chaperones to maintain the misfolded polyQ proteins in a state competent for (proteasomal) degradation, and raises the question of whether the up-regulation of HSP70 alone has any effect on the aggregating polyQ alone.

Implications in terms of neurodegeneration and clinical symptoms
Whereas our data point to an order of events during SCA3 pathology within a single neuron, the implications of the various stages for neuronal dysfunction and clinical symptoms is far from being understood. Based on our data, it seems conceivable, that entrance of ataxin-3 into the nucleus is necessary but not sufficient to cause cell toxicity. Seemingly, additional pathological processes are required to elicit cellular toxicity, such as sequestration of components of the PQC. Furthermore, it is unlikely that this model describes the only pathological pathway at work in SCA3. In the in vitro situation cells displaying only DNS type staining degenerate very readily, so in all likelihood, our model only observes the neurons that mount an initially successful adaptor response, but later in the process suffer from PQC dysregulation instead. Also, protein aggregation may act independently of the PQC system, e.g. by sequestration of known interactors of polyQ proteins and by blockade of axonal transport processes. Likely, SCA3 and possibly other protein aggregation diseases exhibit several pathological pathways, running in parallel, but mounting cumulative stress on the long lived neurons.

Although the observed patterns were highly reproducible amongst 8 different patients that all died at different ages, the limitation of our study, is of course, that it has been done in post-mortem tissue where we only see the end stage situation. Therefore, longitudinal studies in animal models will be required to further test our proposed model. Also, it would be interesting to see whether such a chain of events also applies to other polyQ diseases (e.g. HD or SBMA),
how this compares to some other SCAs that do not exhibit NNI (e.g. SCA2 and SCA6), or at what stages the disease process can be reversed. Furthermore, since p62 is a well known component of autophagic pathways, an investigation of the state of autophagy in human SCA3 tissue would be of interest. What our data again stress is that PQC impairment is a major issue in polyQ diseases and that, likely due to its chronic nature, these systems are not responsive enough to counteract the ongoing, progressive aggregation. Together with the in vitro data and data with models systems, this therefore reemphasizes that boosting the HSR or components thereof could be of potential clinical relevance.
Materials and Methods

Patients and controls
Brains of 8 SCA3 patients (4 males, 4 females, mean age at death 61 ± 15 years) and 5 controls without medical histories of neuropsychiatric diseases (3 males, 2 females, mean age at death 56 ± 26 years) (Supplemental Table 1) were analyzed. Informed consent was obtained from all patients, in accordance with the Medical Ethical Committee of the University Medical Centre Groningen (UMCG) where the autopsies were performed. All brains were fixed for 2 weeks in a 4% phosphate-buffered, aqueous formaldehyde solution (pH 7.4). Tissue blocks from the lower pons were embedded in paraffin and cut into 5 μm thick sections.

Immunohistochemistry
Sections were deparaffinated and rehydrated with a xylol/ethanol sequence. For antigen retrieval, slides were transferred into tris/HCl buffer (pH 9.5), heated 3 x 10 min to 95°C (p62 (rabbit), HSPA1A). For p62 (mouse), 1C2, UBB⁺¹, slides were then transferred into 98% formic acid for 3 min. Endogenous peroxidases were blocked using 0.3% H₂O₂/PBS buffer for 30 min at RT, after which sections were incubated 1 hr at RT with primary antibodies (Supplemental Table 2) diluted in 1% BSA/PBS. Consecutively, the slides were incubated with peroxidase conjugated secondary and tertiary antibodies for 30 min at RT. After staining with 3,3%-DAB (Sigma, St. Louis, USA), sections were counterstained with hematoxylin, dehydrated in ethanol and coverslipped.

For double immunofluorescent staining, sections were treated with 1% sodium borohydride (Sigma, St. Louis, USA) for 10 min to quench autofluorescence. The slides were blocked with 5% normal goat serum (Vector, Burlingame, USA) in PBS containing 0.03% Triton X-100 (Sigma, St. Louis, USA) for 30 min. Next, sections were incubated at 4°C overnight with various primary antibody combinations, washed in PBS and incubated for 3h at RT with goat anti-mouse Alexa488 (Invitrogen, Carlsbad, USA) and goat anti-rabbit Cy3 (Jackson Immunoresearch, West Grove, USA) secondary antibodies. For additional quenching of autofluorescence, sections were treated with 0.05% sudan black solution (Merck, Darmstadt, Germany) for 10 min at RT, washed 8 times in PBS and coverslipped with Mowiol (Sigma, St. Louis, USA).

For quantitative evaluation, 100 neurons per case were scored. Non-parametric, statistical tests were performed with the SPSS version 16.0 software package.
Supplementary Figures

Supplementary Figure 1: Scoring reproducibility. Relative incidence of a given inclusion type as scored by 3 independent researchers. Scoring reproducibility for all stainings, 1C2 (A), Ubiquitin (B) and p62 (C), was highly significant (P values < 0.001). 1C2 and p62, but not ubiquitin staining, (B) showed a distinct distribution of aggregation types.

Supplementary Figure 2: DNAJB1 and HSPA1A stainings in healthy and diseased brain tissue. Photomicrograph of a positive contrast single staining of the pontine nuclei with DNAJB1 (A, B) and HSPA1A (C, D) (DAB staining, brown). There is no discernable difference in neuronal staining intensity between healthy (A, C) and diseased tissue (B, D). Counterstaining with hematoxylen.
Supplemental Figure 3: Co-localization of aggregates with DNAJB1 and sequestration of DNAJB1 into the NNI. Fluorescent double staining with the 1C2 and DNAJB1 antibodies. The antibody labels the majority (85%) of all NNI (arrowheads). In a subset of neurons (6%), DNAJB1 is largely sequestered into the NNI, as can be seen by a lower intensity of somatic staining (arrow) compared to other neurons (asterisks).

Supplemental Figure 4: Co-localization of 1C2 with HSPA1A. Fluorescent double staining demonstrates co-localization of aggregates (1C2: green) with HSPA1A (red) in the NNI of affected neurons.
Supplemental Figure 5. Relative incidence of DNAJB1 sequestration in HSPA1A positive neurons. Graphical representation of the frequency of NNI entrapped DNAJB1 in relation to the staining intensity of HSPA1A (n=105).

Supplemental Table 1: Brain materials from control individuals and SCA3 patients. List of patient number, age at death, gender, diagnosis and CAG repeats, if available. Patients 8 and 9 are as of yet not genotyped, but are clinically diagnosed with SCA3 and from known SCA3 families. Control patients were without history of neurodegenerative or psychiatric diseases.
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*Supplemental Table 2: Information on the primary antibodies.* AR: antibody retrieval (None; MW-: microwave 3x 10 min Tris/HCl pH9; MW+: microwave 3x 10 min Tris/HCl pH9, followed by 3 min 99% formic acid at RT)
Cellular PQC and the evolution of aggregates in SCA3

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List of Abbreviations
transcription frameshift mutant of ubiquitin, Ubiquitin Proteasome System – UPS, University Medical Centre Groningen – UMCG
Spinocerebellar ataxia type 3


Cellular PQC and the evolution of aggregates in SCA3


