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LETTER TO THE EDITOR Open Access

Deposition of mutant ubiquitin in parkinsonism–dementia complex of Guam

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Guam parkinsonism–dementia complex (G-PDC) is an enigmatic neurodegenerative disease that affects the Chamorro residents of the Pacific island of Guam. G-PDC is clinically characterized by progressive cognitive impairment with extrapyramidal signs. Pronounced loss of neurons and abundant neurofibrillary tangles (NFTs) are observed throughout the brain of G-PDC patients [6, 7]. Although several hypotheses have been suggested for the cause of G-PDC, notably genetic predisposition and exposure to neurotoxins (e.g., β-*N*-methylamino-L-alanine (BMAA)), the etiology and pathogenesis remain elusive [10].

A frameshift mutant of ubiquitin, known as ubiquitin-B^{+1} (UBB^{+1}), was previously found to accumulate in the neuropathological hallmarks of Alzheimer's disease and several other disorders, including tauopathies and polyglutamine diseases [1, 3, 12] (Fig. 1a-b). UBB^{+1} is a dose-dependent inhibitor of the ubiquitin-proteasome system (UPS) and its accumulation in cells an indicator of protein quality control failure. Impaired protein homeostasis is a frequent feature of neurodegenerative diseases and we hypothesized that accumulation of UBB^{+1} might also be observed in G-PDC. To test whether UBB^{+1} is detectable in G-PDC brains, immunohistochemical analyses were performed on G-PDC post-mortem brain tissue (Table 1). Immunohistochemistry confirmed the presence of numerous NFTs in G-PDC brains [5] (not shown), as well as other pathology that has been described to occur in G-PDC, i.e., TAR DNA-binding protein 43 (TDP-43)-positive inclusions [5] (Fig. 1f-h). Importantly, our results show that UBB^{+1} is present in G-PDC brains. UBB^{+1} deposits were found specifically in cytoplasm of pyramidal neurons and glia (astrocytes in the alveus and stratum oriens) in Ammon's horn, showing a granular and tangle-like pattern of distribution (Fig. 1c-e). UBB^{+1} was not detected in young control brains (n = 2, non-Guamanian cases, ages: 52 and 59 years old) [8]. Aggregate structures containing distinct components of the UPS, i.e., the deubiquitinating enzyme (DUB) ubiquitin C-terminal hydrolase L1 (UCH-L1) [9] (Fig. 1i-k) and the proteasomal ATPase subunit Rpt3/S6b [13] (Fig. 1l-n), were also present in these brains.

This demonstration of UBB^{+1}-immunoreactivity and accumulation of particular UPS components in G-PDC brains (n = 6) might have important implications for understanding of the pathological mechanisms underlying the disease. UBB^{+1} has previously been shown to induce neuronal defects in in vitro and in vivo experimental models: long-term UPS inhibition due to UBB^{+1} expression causes memory deficits and central breathing dysfunction in mice [4, 8, 11]. In addition, UBB^{+1} might act as a modifier of other pathology in G-PDC. For example, UBB^{+1} may enhance the aggregation and cellular toxicity of the RNA-binding protein TDP-43 through interfering with its degradation. It is striking that UBB^{+1} accumulates in glial cells in G-PDC, because similar glial inclusions have been reported in progressive supranuclear palsy (PSP) [3], a disease that displays some similar topography of neurofibrillary degeneration [10]. Recognition of common mechanistic themes shared by neurodegenerative disorders, such as dysfunctional (ubiquitin-dependent) protein degradation and proteotoxic stress, may help in identifying therapeutic targets that prevent neurodegeneration. It will be interesting to investigate the potential contribution of disrupted proteostasis and UBB^{+1} to G-PDC in more detail in future studies.

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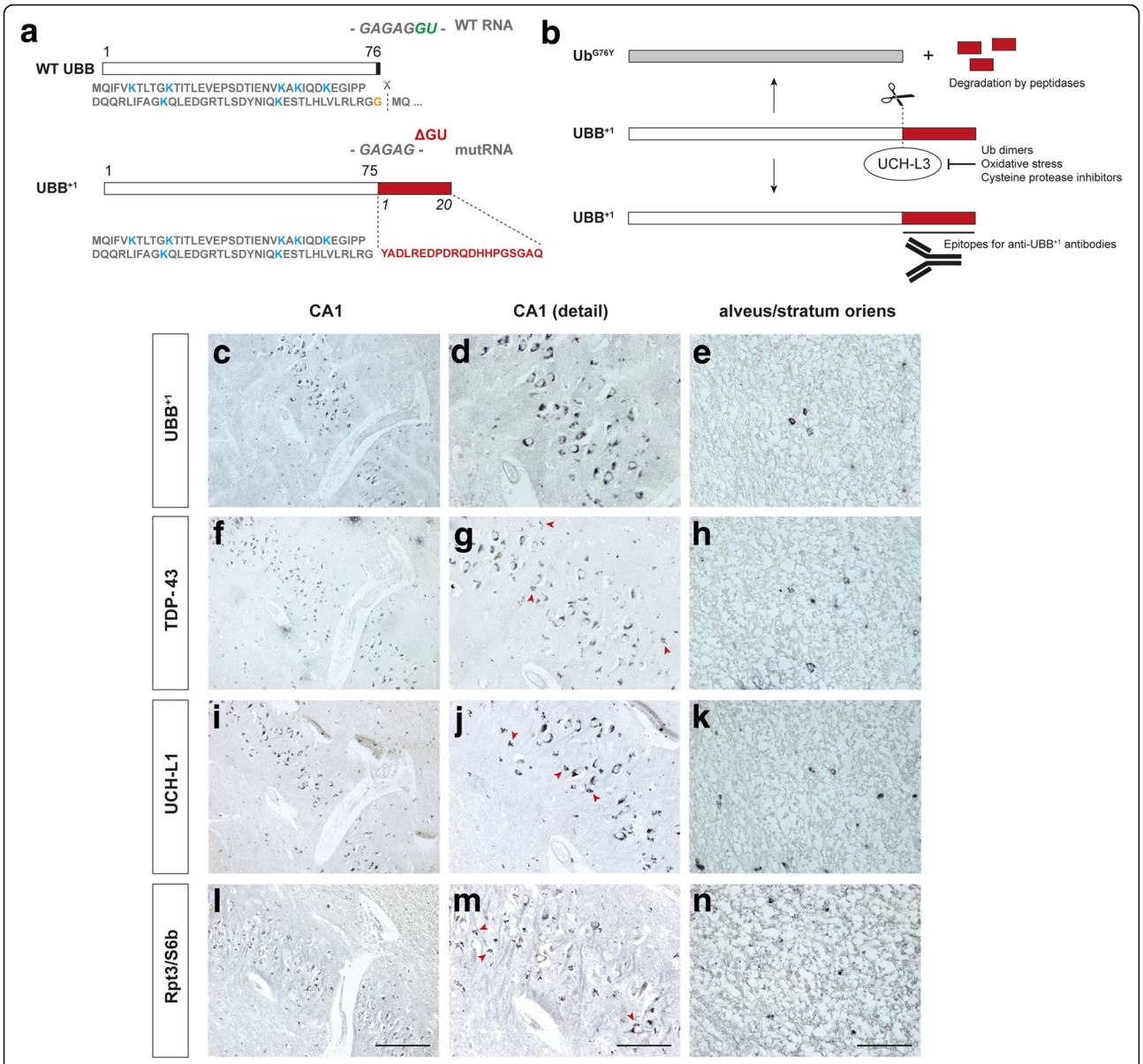

Fig. 1 Mutant ubiquitin (UBB^{+1}) is deposited in Guam parkinsonism–dementia complex (G-PDC) brains. **a** UBB^{+1} is generated through "molecular misreading", a type of transcriptional mutagenesis. The resulting unfaithful RNA messengers can generate abnormal proteins with cytotoxic properties. **b** UBB^{+1} contains an extended C-terminal domain, which can be recognized by anti-UBB^{+1} antibodies. Deubiquitating enzymes (DUBs) can hydrolyze this extended C-terminus. However, inhibition of these DUBs, e.g., by oxidative stress conditions, prevents this cleavage, preserving the epitope [2]. **c-e** Immunostaining for UBB^{+1} (Ubi2A, 1:400, Dr. F.W. van Leeuwen [3]) reveals many cytoplasmic structures in neurons and glial cells (i.e., astrocytes in the alveus and stratum oriens) of the hippocampus. **f-h** Additionally, cytoplasmic TAR DNA-binding protein 43 (TDP-43) aggregates can be observed in the same cell types (mouse anti-TDP-43, 1:1000, Abnova). **i-k** Aggregates containing ubiquitin C-terminal hydrolase L1 (UCH-L1) (rabbit anti-UCH-L1, 1:500, Biomol), a DUB, and **l-n** Rpt3/S6b (rabbit anti-Rpt3, 1:400, Biomol), a proteasomal subunit [13], are also found in G-PDC. Several immunoreactive structures show a granular staining pattern (arrowheads). All immunostainings were carried out on 6 μm thick formalin-fixed, paraffin-embedded sections. Panels **c-n** all show representative images of G-PDC hippocampi (adjacent sections from subject #2, Table 1). *Scale bars* 200 μm (**c, f, i, l**), 100 μm (**d, g, j, m**), and 50 μm (**e, h, k, n**)

Table 1 Description of the subjects

Subject	Sex	Age of death (years)	Age of onset (years)	Disease duration (months)	Brain weight (g)	Post-mortem delay	Cause of death	UBB^{+1}
1	F	51	42	116	850	3 h	perforated gastric ulcer	++
2	M	64	58	72	1275	7 h	pulmonary atelectasis	++++
3	M	52	42	126	1025	4 h	bronchopneumonia	++
4	M	56	46	126	1235	< 10 h	bronchopneumonia	+++
5	F	51	46	59	1135	8 h	bronchopneumonia	++
6	M	84	80	50	1100	14 h	bronchopneumonia	++

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Ethics approval and consent to participate
This study was conducted with the approval of the Ethical Committee of Shinshu University School of Medicine (No. 1565).

Competing interests
The authors declare that they have no competing interests.

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