SCA17 repeat expansion: Mildly expanded CAG/CAA repeat alleles in neurological disorders and the functional implications

Chiung-Mei Chen a,1, Li-Ching Lee b,1, Bing-Wen Soong c, Hon-Chung Fung a, Wen-Chuin Hsu a, Pei-Ying Lin b, Hui-Ju Huang b, Fen-Lin Chen b, Cheng-Yueh Lin b, Guey-Jen Lee-Chen b,⁎, Yih-Ru Wu a,⁎

a Department of Neurology, Chang Gung Memorial Hospital and Chang-Gung University College of Medicine, Taipei, Taiwan
b Department of Life Science, National Taiwan Normal University, Taipei, Taiwan
c Department of Neurology, National Yang-Ming University School of Medicine, The Neurological Institute, Taipei Veterans General Hospital, Taipei, Taiwan

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ABSTRACT

Background: Spinocerebellar ataxia type 17 (SCA17) involves the expression of a CAG/CAA expansion mutation in the gene encoding TATA-box binding protein (TBP), a general transcription initiation factor. The spectrum of SCA17 clinical presentation is broad.

Methods: We screened for triplet expansion in the TBP gene in Taiwanese Parkinson’s disease (PD), Alzheimer’s disease (AD) and atypical parkinsonism and investigated the functional implication of expanded alleles using lymphoblastoid cells as a model.

Results: A total of 6 mildly expanded alleles (44–46) were identified in patients group. The frequency of the individuals carrying expanded alleles in PD (3/602 [0.5%]), AD (2/245 [0.8%]) and atypical parkinsonism (1/44 [2.3%]) is not significant as compared to that in the control subjects (0/644 [0.0%]). In lymphoblastoid cells, HSPAS, HSPA8 and HSPB1 expression levels in cells with expanded TBP were significantly lower than that of the control cells. Although not significantly, the levels of PARK7 protein isoforms 6.1 and 6.4 are notably increased in SCA17 lymphoblastoid cells. Treatment of TBH (terti-butyl hydroperoxide) significantly increases cell death in the cells with mildly expanded TBP.

Conclusions: Our findings expand the spectrum of SCA17 phenotype and may contribute to our understanding of the disease.

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1. Introduction

Spinocerebellar ataxia type 17 (SCA17) is an autosomal dominant ataxia caused by an expanded polyglutamine (polyQ) in a general transcription factor, the TATA-box binding protein (TBP) [1,2]. In addition to progressive gait and limb ataxia, the broad phenotypic spectrum of this rare disorder includes seizure, cognitive dysfunctions, psychiatric symptoms, and pyramidal and extrapyramidal features such as spasticity, dystonia, chorea, and parkinsonism (review in [3]). Unrelated expanded alleles found in familial and sporadic ataxia patients range from 43–66 repeats [4,5] as opposed to 25–42 repeats in the general population [6]. Reduced penetrance was observed for alleles with 44–47 repeats [7]. Small TBP expansions accounting for a small proportion of patients with Parkinson’s disease (PD)-like, multiple system atrophy, Alzheimer’s disease (AD)-like and Huntington’s disease-like phenotypes have also been shown [8–12].

The transcriptional involvement of TBP is well characterized [13]. Nevertheless, how the polyQ domain contributes to the normal function of proteins and the expanded polyQ induces selective neuropathology remain unclear. A mechanism for aberrant polyQ gain of function and increased Cre-dependent transcriptional activity in SCA17 has been suggested [14]. Studies of the impact of polyQ expansion on TBP function further revealed that expanded polyQ tracts reduce TBP dimerization and DNA binding, but enhance the interaction of TBP with TFIIB to down-regulate heat shock 27 kDa protein 1 (HSPB1) expression [15,16]. Our previous proteomics study using a cellular model of SCA17 has revealed altered expression of heat shock 70 kDa protein 5 (HSPA5), heat shock 70 kDa protein 8 (HSPA8) and Parkinson’s disease 7 (PARK7), suggesting that impaired protein folding may contribute the cell dysfunction of SCA17 [17].

Although the pathology of SCA is mainly in cerebellum, most SCA genes are ubiquitously expressed in central nervous system and
peripheral tissues including peripheral lymphocytes. Lymphoblastoid cells are frequently used as a model to study SCA [18,19]. Although our previous study has shown mildly expanded SCA17 CAG repeats in one PD and two AD patients [10,11], in this study, we examined the length of the SCA17 repeats in a larger cohort of ethnic Chinese PD, AD and atypical parkinsonism in Taiwan. Furthermore, we investigated if the HSPA5, HSPA8, HSPB1 and PARK7 expression levels are altered and if sensitivity to tert-butyl hydroperoxide (TBH), an oxidant, is increased in lymphoblastoid cells carrying SCA17 mildly expanded repeat tracts.

2. Materials and methods

2.1. Subjects

The study group comprised 602 sporadic PD patients (45.6% females, aged 69.4±10.7 years), 245 sporadic AD patients (62.4% females, aged 75.8±8.6 years) and 44 atypical parkinsonism patients (46.7% females, aged 69.9±10.3 years) recruited from the outpatient clinic of Chang Gung Memorial Hospital. All PD patients were diagnosed according to the published criteria [20]. Probable AD was diagnosed by consensus based on the criteria for probable AD of the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer’s Disease and Related Disorders Association (NINCDS–ADRDA) [21]. Subjects with atypical parkinsonism include subgroups of corticobasal degeneration, progressive supranuclear palsy, dementia with Lewy bodies and multiple system atrophy [22]. A group of 644 subjects (47.7% females, aged 59.4±14.6 years) was enrolled as the normal control group. The age difference was statistically significant between cases and controls. All the cases and controls in the study were Han Chinese in Taiwan and not related to each other. Each control was interviewed by one of the neurologists (Y-R Wu and C-M Chen) to rule out the possibility of parkinsonism, dementia, ataxia, and other neurological diseases. Each subject was informed of the study goals, and all examinations were performed after obtaining informed consent.

2.2. Genetic analysis of SCA17 repeats

DNA was extracted from leukocytes, and the SCA17 CAG/CAA repeats were determined by polymerase chain reaction (PCR) amplification [1] and PCR products resolved on a linear polyacrylamide gel using an automated MegaBACE Analyzer. Allele sizes were determined by comparing the migration relative to the standard molecular weights. DNA sequencing was performed to assess the repeat number, size, and gender. The expanded alleles in all PD, AD and atypical parkinsonism patients were typically confirmed by DNA sequencing.

2.3. Lymphoblastoid cell lines and cell viability assay

Lymphoblastoid cell lines from 3 SCA patients (P1–P3, 55, 44 and 46 repeats), one PD patient (P4, 44 repeats), 1 AD patient (P5, 46 repeats) and 1 atypical parkinsonism patient (P6, 46 repeats) as well as age- and gender-matched normal controls (C1–C6, 36 repeats) were established (Food Industry Research and Development Institute, Taiwan) after obtaining informed consent. The 3 SCA patients were recruited from Taipei Veterans General Hospital. The clinical features of the three SCA patients were mainly ataxic gait with or without dementia. The rest patients and controls were recruited from Chang Gung Memorial Hospital. Cells were maintained in RPMI 1640 medium (Gibco) containing 10% FCS.

To evaluate the cell viability, the cultured cell suspensions (10^5 in 100 µl) in medium with or without 10% FCS were treated with TBH (10–20 µmol/l). After 24 or 48 h, 20 µl 0.4% trypan blue (Gibco) was mixed with 20 µl cell suspension sampled from each well for 1 h. Cell viability was determined by light microscopy. Cells that excluded trypan blue were considered viable and counted in quadruplicate.

2.4. Western blot analysis

Total protein lysate from lymphoblastoid cells was prepared using lysis buffer (in PBS) containing 5% glycerol, 0.5% Triton X-100, 1 mmol/l dithiothreitol, and protease inhibitor cocktail (Sigma). Protein lysate (25 µg) was separated in 10% SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and blotted onto nitrocellulose membranes by reverse electrophoresis. After blocking, the membrane was stained with antibody to TBP (1:3000 dilution, Abcam), HSPA5, HSPA8 (1:500 dilution, Santa Cruz Biotechnology), HSPA5, HSPA8 (1:500 dilution, Santa Cruz Biotechnology), or C4 (actin) (1:10,000 dilution, Chemicon). The immune complexes were detected using horseradish peroxidase-conjugated donkey anti-goat (Santa Cruz Biotechnology) or goat anti-mouse (Jackson Immunoresearch) IgG antibody (1:10,000 dilution) and chemiluminescent substrate.

2.5. Two-dimensional gel electrophoresis and immunoblot analysis

Protein extracts were prepared from lymphoblastoid cells as described [17]. Protein samples (300 µg) were first separated using Immobiline DryStrip (7 cm, pH 3–10) (GE Healthcare) and further separated by a 12.5% SDS-PAGE. The blotting membranes were stained with antibody to PARK7 (1:10,000 dilution, Chemicon) and immune complexes detected using horseradish peroxidase-conjugated goat anti-rabbit IgG antibody (1:10,000 dilution, Rockland) and chemiluminescent substrate.

3. Results

3.1. Frequency distributions of SCA17 repeat lengths

PCR amplification of the SCA17 locus yielded a pattern of 2 distinct alleles in 63% of the cases analyzed. Fig. 1 shows the distributions of the SCA17 repeat lengths in both alleles in the 602 PD patients, 245 AD patients, 44 atypical parkinsonism patients, and 644 normal control subjects. The controls were found to be in the Hardy–Weinberg equilibrium (\( \chi^2 = 3.27, df = 13, P = 0.997 \)). At the SCA17 locus, CAG repeat length varied from 28 to 43 in the control group and from 27 to 46 in the patient group. In both control and patient groups, the most common length was 36 repeats (52–56%). Mildly expanded CAG repeats were found in three PD (44, 44 and 46 repeats), two AD (44 and 46 repeats) and one atypical parkinsonism (46 repeats) patients. The frequency of individuals carrying mildly expanded SCA17 alleles in PD (3/602 [0.5%]; \( P = 0.112 \)), AD (2/245 [0.8%]; \( P = 0.076 \)) or atypical parkinsonism (1/44 [2.3%]; \( P = 0.064 \)) was not significantly different from that in the control subjects (0/644 [0%]).

3.2. Clinical analysis of patients with SCA17 expansions

The clinical features and repeat sequences of one PD (77/F, 46 repeats) and two AD (59/F, 45 repeats; 76/F, 46 repeats) patients have been described previously [10,11]. Patient H1288 (48/M, PD, case 1) had 44 repeats. The CAG expansion number in patient H1173 (62/F, PD, case 2) was 44. Patient H1387 (76/F, DLBD, case 3) had 46 repeats. The clinical features and the sequences of mildly expanded alleles of the newly identified patients with TBP expansions are displayed in Table 1. The relatives of H1288 and H1387 were not available for the
genetic test. The son of H1173 also has the same mildly expanded SCA17 sequence as his mother, but neurological and physical examination revealed no ataxic gait, or any neurological abnormality. Interestingly, 99mTc-TRODAT-1 SPECT showed a prominent decrease in striatal DAT binding in two PD patients (H1288 and H1173). In addition to SCA17 expansion, SCA8 CAG expansion was also seen in patients H1173 (84 CTA/CTG repeats) and H1387 (97 CTA/CTG repeats) [24].

3.3. Lymphoblastoid cell lines establishment and TBH sensitivity

Lymphoblastoid cells from six patients with TBP expansions, including 3 SCA (P1, 28/F, 36/55 repeats; P2, 78/F, 33/46 repeats; P3, 80/M, 37/44 repeats), 1 AD (P4, 76/F, 36/46 repeats), 1 PD (P5, 62/F, 36/44 repeats), 1 DLBD (P6, 76/F, 36/46 repeats), and 6 age- and gender-matched normal controls (C1, 23/F; C2, 70/F; C3, 68/M; C4, 70/F; C5, 63/F; C6, 68/F; 36/36 repeats) were established. As shown in Fig. 2A, Western blot analysis using TBP antibody detected a single band (non-expanded repeats) protein in all six control cell lines, whereas a larger TBP protein carrying 44–55 repeats and a small TBP protein carrying 33–37 repeats were detected in cell lines from patients with TBP expansions.

The sensitivity of TBP expansions to TBH, a mild oxidative reagent, was examined using lymphoblastoid cell lines from 3 SCA patients (P1–P3) and 3 normal controls (C1–C3). As summarized in Fig. 2B, with 10–20 µmol/l TBH treatment for 0, 24 and 48 h, the percentages of cells that retained trypan blue were 7.8–9.3% (0 h), 11.6–12.2% (24 h), 12.7–14.8% (48 h) for cells with TBP expansion (P1–P3) and 7.9–9.7% (0 h), 10.6–11.7% (24 h), 11.6–12.4% (48 h) for cells without expansion.

Table 1
Clinical features and repeat sequences in PD (cases 1–2) and atypical parkinsonism (case 3) patients carrying TBP expansion allele.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Sex, onset age, age at examination (years)</th>
<th>Clinical features at examination</th>
<th>Image studies</th>
<th>Repeat sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case 1 (H1288)</td>
<td>M, 46, 48</td>
<td>Asymmetric resting tremor, bradykinesia, and rigidity, levodopa responsive</td>
<td>Brain MRI: normal, TRODAT: asymmetric reduction of activity of DAT in the bilateral striatum</td>
<td>(CAG)3(CAA)3(CAG)8CAACAGCAA(CAG)25CAACAG</td>
</tr>
<tr>
<td>Case 2 (H1173)</td>
<td>F, 40, 62</td>
<td>Asymmetric resting tremor, bradykinesia, and rigidity, levodopa responsive; levodopa-induced dyskinesia; dementia at late disease stage</td>
<td>Brain MRI: normal, TRODAT: asymmetric reduction of activity of DAT in the bilateral striatum</td>
<td>(CAG)3(CAA)3(CAG)8CAACAGCAA(CAG)25CAACAG</td>
</tr>
<tr>
<td>Case 3 (H1387)</td>
<td>F, 75, 76</td>
<td>Symmetric resting tremor, bradykinesia, and rigidity; dementia at early disease stage; visual hallucination</td>
<td>Brain CT: moderate cerebral atrophy</td>
<td>(CAG)3(CAA)3(CAG)8CAACAGCAA(CAG)25CAACAG</td>
</tr>
</tbody>
</table>

F: female; M: male; TRODAT: 99mTc-TRODAT-1 SPECT; DAT: dopamine transporter.

TBP expansion (C1–C3). Although the difference between patient and control groups for the same treatment concentration and time was not significant ($P=0.109$–$0.686$), the increase of cell death after 10 µmol/l TBH treatment for P1 from 7.8% to 9.8% (24 h) and to 10.2% (48 h), P2 from 9.0% to 10.6% (24 h) and to 11.9% (48 h) and P3 from 9.3% to 11.7% (48 h) was significant ($P=0.046$–$0.004$) as compared to untreated cells. For control cell lines C1–C3, the increase of cell death was not significant ($P>0.05$) with the same 10 µM TBH treatment. With 20 µM TBH treatment, the increase of cell death for P1 from 7.8% to 11.6% (24 h) and to 12.7% (48 h), P2 from 9.0% to 12.1% (24 h) and to 14.8% (48 h) and P3 from 9.3% to 12.2% (24 h) and to 13.5% (48 h) was significant ($P=0.037$–$0.000$) as compared to untreated cells. For control cell lines C1–C3, the increase of cell death was only significant ($P=0.015$–$0.004$) with 20 µmol/l TBH treatment for 48 h.

3.4. Analysis of HSPA5, HSPA8, HSPB1 and PARK7 expression

As reduced expression of several heat shock proteins was implicated in SCA17 pathogenesis [15–17], we examined the expression level of HSPA5, HSPA8 and HSPB1 using Western blotting and appropriate antibodies (Fig. 3A). When the expressed level in one of the control in each blot (C1 and C4) was set as 100% HSPA5 protein expression in lymphoblastoid cells expressing TBP-Q44–55 (P1–P6) was significantly reduced when compared to the lymphoblastoid cells expressing TBP-Q26 (C1–C6) (median: 76% vs. 100%, range: 71–81% vs. 90–117%, $P=0.001$). Similarly, HSPA8 and HSPB1 protein expression in lymphoblastoid cells expressing expanded TBP were also significantly reduced when compared to the lymphoblastoid cells expressing normal TBP (for HSPA8, median: 85% vs. 100%, range: 80–89% vs. 87–115%, $P=0.010$; for HSPB1, median: 75% vs. 101%, range: 58–89% vs. 87–109%, $P=0.001$). The relative HSPA5, HSPA8 and HSPB1 protein levels in lymphoblastoid cells from controls and patients with TBH expansions are shown in Fig. 3B.

As different PARK7 isoforms exist [25], we performed comparative high resolution, two-dimensional gel electrophoresis experiments on protein extracts from three SCA17 patients and age- and gender-matched controls to investigate the expression and posttranslational modification of PARK7. Immunoblot analysis with PARK7 antibody revealed the presence of at least four distinct isoforms that have the same apparent molecular mass of 20 kDa but different isoelectric points (5.7, 5.8, 6.1 and 6.4, respectively; Fig. 3C). The predominant form of PARK7 is the 20-kDa/pl 6.4 isoform. Although not significant, the increase level of PARK7 at pl 6.1 (median: 301% vs. 100%, range: 160–387% vs. 89–111%, $P=0.104$) and pl 6.4 (median: 193% vs. 100%, range: 120–246% vs. 62–127%, $P=0.116$) isoforms in SCA17 lymphoblastoid cells was notable.

4. Discussion

This study examined the length of the SCA17 repeats in Taiwanese patients with PD, AD and atypical parkinsonism, and in normal control subjects. Mildly expanded SCA17 alleles (44 and 46 repeats) were found in 6 patients, including 3 PD, 2 AD and 1 atypical parkinsonism (Fig. 1). Recently, Kim and colleagues have also reported 8 PD patients carrying 43–45 SCA17 repeats, which is in accordance with our finding, suggesting that low-range SCA17 expansions tend to have a parkinsonian phenotype [12]. Although the possibility of reduced penetrance of TBP 44–47 repeats exists [7], and the possibility of coincidence of the mildly expanded CAG repeats with PD, AD or atypical parkinsonism cannot be completely excluded, the reported SCA17 cases with atypical parkinsonism and PD [12,26] and our results strengthen the previous observation [10,11] that SCA17 CAG repeat expansion may be a rare cause of PD, AD and atypical parkinsonism.

A role for oxidative damage in polyQ diseases is gathering increasing experimental support and oxidative stress may be a predisposing factor to the late-onset feature of SCA17. Thus we established lymphoblastoid cell lines and used a mild oxidative reagent TBH [27] and trypan blue exclusion assay to assess the oxidative vulnerability of cells carrying expanded TBP. We observed increased susceptibility to oxidative stress that leads to increased cell death in SCA17 lymphoblastoid cells carrying TBP expansion (P1, 55 repeats; P2, 46 repeats;
However, the increased vulnerability and cell death to oxidative stress did not correlate well with the length of polyQ, which may be due to the prominent age difference and/or other genetic variations among the three patients.

As a basal transcription factor, TBP is involved in both TATA and TATA-less promoter sites. Transcriptional dysfunction of enhancing TFIIB–TBP interaction to down-regulate the small heat shock protein HSPB1 (HSP27) expression has been reported\[15,16\]. Our proteomics study of a SCA17 cellular model also demonstrated reduced expression of heat shock protein HSPA5 and HSPA8\[17\]. The HSPB1 is involved in stress resistance. In a HD cellular model, HSPB1 was proposed to suppress polyQ induced reactive oxygen species that contributed to cell death\[28\]. Defects in the HSPB1 gene are a cause of 2 related peripheral neuropathies, Charcot-Marie-Tooth disease type 2 and distal hereditary motor neuropathy\[29\]. The HSPAS (also known as 78 kDa glucose-regulated heat shock protein GRP-78) is involved in the folding and assembly of proteins in the endoplasmic reticulum. Abnormal protein folding due to decreased rate of HSPA5 hydrolysis and disturbed SIL1-HSPA5 interaction is the cause of Marinesco–Sjogren syndrome that is characterized by ataxia, progressive myopathy and cataract\[30\]. The HSPA5 encodes a constitutively expressed 70 kDa heat shock cognate protein (HSC70) binding to nascent polypeptides to facilitate correct folding. Immunohistochemical studies on HSPA5 using autopsied brains from patients with multiple system atrophy suggest that HSPA5 may be associated with the pathogenesis of multiple system atrophy\[31\]. The above evidence suggests that altered expression of HSPB1, HSPA5, and HSPA8 may contribute to neuronal dysfunction in SCA17. To assess the roles of these chaperones in SCA17, we examined the expression levels of HSPA5, HSPA8 and HSPB1 in lymphoblastoid cells carrying expanded TBP. We have shown that HSPB1, HSPA5 and HSPA8 expression levels were significantly reduced in transformed lymphoblastoid cells from patients with mild TBP expansion (Fig. 3B). While direct evidence that HSPB1, HSPA5 and/or HSPA8 contribute to the pathogenesis of SCA17 is not provided in this manuscript, in SCA17 transgenic mice, down-regulated HSPB1 due to decreased general transcription factor TFIIB occupancy of the \(Hspb1\) promoter has been shown, and overexpression of HSPB1 or TFIIB alleviated mutant TBP-induced neuritic defects\[15\]. Furthermore, an expanded polyQ domain that can inhibit the intrinsic association of TBP with TATA-box DNA \textit{in vivo} has been shown to influence TBP-dependent gene transcription\[16\]. Although whether the reduced HSPA5 or HSPA8 gene expression is TBP-mediated is not known, the previous finding together with our results and reports that the reduced expression of HSP27 and/or HSP70 in lymphoblastoid cells from patients with SCA3 and SCA7\[18,19\] supports the proposal that reduction of HSPB1, HSPA5 and/or HSPA8 may contribute to the neuronal dysfunction of polyQ-mediated SCA.

In addition to HSPB1, HSPA5 and HSPA8 chaperones, we also examined the up-regulated PARK7, a causative gene of early-onset PD. PARK7, also known as DJ-1, has a role in the anti-oxidative stress reaction to prevent cell death\[32\]. Increased expression of PARK7 in HEK-293 cell models expressing expanded TBP has been shown in our previous study\[17\]. However, maybe due to the expression level difference (constitutive vs. induced) between the lymphoblastoid and

HEK-293 cell models and/or the small difference in the polyglutamine length (44–55 repeats in SCA17 lymphoblastoid cells vs. 61 repeats in HEK-293 cells) compared to the upper limit of normal repeat range, the observed increased expression of PARK7 in SCA17 lymphoblastoid cells with expanded TBP was not significant (Fig. 3C). Our study however has a couple of limitations. One of them is the small number of cell lines used, because both inter and intra-individual variability could be a confounding variable. Another limitation is that the possibility of co-incidence of the mildly expanded CAG repeats with PD, AD or atypical parkinsonism cannot be completely excluded. However, there are two lines of evidence to support that the mildly expanded CAG repeats in PD, AD or atypical parkinsonism are pathogenic. One is that there is no such mildly expanded CAG repeat in 644 controls of our study. Another one comes from that in the study of Kim et al.: they have shown that CAG expansion in the SCA17 gene ranged from 43 to 46 in 10 parkinsonian patients, suggesting low-range expansions tend to cause the parkinsonian phenotype [12]. Furthermore, they have shown decreased striatal dopamine transporter binding in 4 control individuals with 42 repeats. Our cell models may be valuable because they shed insight into the pathogenesis and provide a basis for developing therapeutic interventions for this disease.

Acknowledgments

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