a potent pathogenic marker of SBMA, the 6MWT might be used in combination with other biomarkers in order to determine response to therapeutics.[6]

In conclusion, our observations suggest that the 6MWT is a reliable biomarker to quantify exercise capacity in patients with neuromuscular disorders such as SBMA.

We thank Dr. Hiroshi Yatsuya for statistical advice. This work was supported by a Center-of-Excellence (COE) grant from the Ministry of Education, Culture, Sports, Science and Technology of Japan, grants from the Ministry of Health, Labor and Welfare of Japan, a grant from Japan Intractable Diseases Research Foundation and the Program for Improvement of Research Environment for Young Researchers from Special Coordination Funds for Promoting Science and Technology (SCF) commissioned by the Ministry of Education, Culture, Sports, Science and Technology of Japan.

REFERENCES

1. Adachi H, Katsuno M, Minamiyama M, Waza M, Sang C, Nakagomi Y, et al. Widespread nuclear and cytoplasmic accumulation of mutant androgen receptor in SBMA patients. Brain 2005;128:659–670.
2. Andersson C, Asztalos L, Mattsson E. Six-minute walk test in adults with cerebral palsy. A study of reliability. Clin Rehabil 2006;20:488–495.
3. Andrew SE, Goldberg YP, Hayden MR. Rethinking genotype and phenotype correlations in polyglutamine expansion disorders. Hum Mol Genet 1997;6:2005–2010.
4. ATS statement: guidelines for the six-minute walk test. Am J Respir Crit Care Med 2002;166:111–117.
5. Atsuta N, Watanabe H, Ito M, Banno H, Suzuki K, Katsuno M, et al. Natural history of spinal and bulbar muscular atrophy (SBMA): a study of 223 Japanese patients. Brain 2006;129: 1446–1455.
6. Banno H, Adachi H, Katsuno M, Suzuki K, Atsuta N, Watanabe H, et al. Mutant androgen receptor accumulation in spinal and bulbar muscular atrophy scrotal skin: a pathogenic marker. Ann Neurol 2006;59:520–526.
7. Barbeau H, Elashoff R, Deforge D, Ditunno J, Saulino M, Dobkin BH. Comparison of speeds used for the 15.2-meter and 6-minute walks over the year after an incomplete spinal cord injury: the SCILT Trial. Neurorehabil Neural Repair 2007;21:302–306.
8. Canning CG, Ada L, Johnson JJ, Mcwhirter S. Walking capacity in mild to moderate Parkinson's disease. Arch Phys Med Rehabil 2006;87:371–375.
9. Daly JJ, Roenigk K, Holcomb J, Rogers JM, Butler K, Gansen J, et al. A randomized controlled trial of functional neuromuscular stimulation in chronic stroke subjects. Stroke 2006; 37:172–178.
10. Doyu M, Sobue G, Mukai E, Kachi T, Yasuda T, Mitsuma T, et al. Severity of X-linked recessive bulbospinal neuronopathy correlates with size of the tandem CAG repeat in androgen receptor gene. Ann Neurol 1992;32:707–710.
11. Duncan P, Richards L, Wallace D, Stoker-Yates J, Pohl P, Luchies C, et al. A randomized, controlled pilot study of a home-based exercise program for individuals with mild and moderate stroke. Stroke 1998;29:2055–2060.
12. Eng JJ, Chu KS, Dawson AS, Kim CM, Hepburn KE. Functional walk tests in individuals with stroke: relation to perceived exertion and myocardial exertion. Stroke 2002;33: 756–761.
13. Fischbeck KH. Kennedy disease. J Inherit Metab Dis 1997;20: 152–158.
14. Gatchel JR, Zoghbi HY. Diseases of unstable repeat expansion: mechanisms and common principles. Nat Rev Genet 2005;6:743–755.
15. Gonzalez H, Sunnerhagen KS, Sjoberg I, Kaponides G, Olsson T, Borg K. Intravenous immunoglobulin for post-polio syndrome: a randomised controlled trial. Lancet Neurol 2006;5: 493–500.
16. Gylfadottir S, Dallimore M, Dean E. The relation between walking capacity and clinical correlates in survivors of chronic spinal poliomyelitis. Arch Phys Med Rehabil 2006;87:944–952.
17. Harmatz P, Giugliani R, Schwartz I, Guffon N, Teles EL, Miranda MC, et al. Enzyme replacement therapy for mucopolysaccharidosis VI: a phase 3, randomized, double-blind, placebo-controlled, multinational study of recombinant human N-acetylgalactosamine 4-sulfatase (recombinant human arylsulfatase B or rhASB) and follow-on, open-label extension study. J Pediatr 2006;148:533–539.
18. Katsuno M, Adachi H, Doyu M, Minamiyama M, Sang C, Kobayashi Y, et al. Leuprorelin rescues polyglutamine-dependent phenotypes in a transgenic mouse model of spinal and bulbar muscular atrophy. Nat Med 2003;9:768–773.
19. Katsuno M, Sang C, Adachi H, Minamiyama M, Waza M, Tanaka F, et al. Pharmacological induction of heat-shock proteins alleviates polyglutamine-mediated motor neuron disease. Proc Natl Acad Sci U S A 2005;102:16801–16806.
20. Kennedy WR, Alter M, Sung JH. Progressive proximal spinal and bulbar muscular atrophy of late onset. A sex-linked recessive trait. Neurology 1968;18:671–680.
21. Kierkegaard M, Tollback A. Reliability and feasibility of the six minute walk test in subjects with myotonic dystrophy. Neuromuscul Disord 2007;17:943–949.
22. La Spada AR, Wilson EM, Lubahn DB, Harding AE, Fischbeck KH. Androgen receptor gene mutations in X-linked spinal and bulbar muscular atrophy. Nature 1991;352:77–79.
23. Mannerkorpi K, Svantesson U, Broberg C. Relationships between performance-based tests and patients' ratings of activity limitations, self-efficacy, and pain in fibromyalgia. Arch Phys Med Rehabil 2006;87:259–264.
24. Minamiyama M, Katsuno M, Adachi H, Waza M, Sang C, Kobayashi Y, et al. Sodium butyrate ameliorates phenotypic expression in a transgenic mouse model of spinal and bulbar muscular atrophy. Hum Mol Genet 2004;13:1183–1192.
25. Noonan VK, Dean E, Dallimore M. The relationship between self-reports and objective measures of disability in patients with late sequelae of poliomyelitis: a validation study. Arch Phys Med Rehabil 2000;81:1422–1427.
26. Papapetropoulos T, Panayiotopoulos CP. X-linked spinal and bulbar muscular atrophy of late onset (Kennedy-Stefanis disease?). Eur Neurol 1981;20:485–488.
27. Savci S, Inal-Ince D, Arikan H, Guclu-Gunduz A, Cetisli-Korkmaz N, Armutlu K, et al. Six-minute walk distance as a measure of functional exercise capacity in multiple sclerosis. Disabil Rehabil 2005;27:1365–1371.
28. Sobue G, Hashizume Y, Mukai E, Hirayama M, Mitsuma T, Takahashi A. X-linked recessive bulbospinal neuronopathy. A clinicopathological study. Brain 1989;112(Pt 1):209–232.
29. Solway S, Brooks D, Lacasse Y, Thomas S. A qualitative systematic overview of the measurement properties of functional walk tests used in the cardiorespiratory domain. Chest 2001; 119:256–270.
30. Sperfeld AD, Karitzky J, Brummer D, Schreiber H, Häussler J, Ludolph AC, et al. X-linked bulbospinal neuronopathy: Kennedy disease. Arch Neurol 2002;59:1921–1926.
31. Suzuki K, Katsuno M, Banno H, Takeuchi Y, Atsuta N, Ito M, et al. CAG repeat size correlates to electrophysiological motor and sensory phenotypes in SBMA. Brain 2008;131:229–239.
32. Tanaka F, Doyu M, Ito Y, Matsumoto M, Mitsuma T, Abe K, et al. Founder effect in spinal and bulbar muscular atrophy (SBMA). Hum Mol Genet 1996;5:1253–1257.

33. Waza M, Adachi H, Katsuno M, Minamiyama M, Sang C, Tanaka F, et al. 17-AAG, an Hsp90 inhibitor, ameliorates polyglutamine-mediated motor neuron degeneration. Nat Med 2005;11:1088–1095.

34. Wraith JE, Clarke LA, Beck M, Kolodny EH, Pastores GM, Muenzer J, et al. Enzyme replacement therapy for mucopolysaccharidosis I: a randomized, double-blinded, placebo-controlled, multinational study of recombinant human alpha-L-iduronidase (laronidase). J Pediatr 2004;144:581–588.

35. Yamada M, Inaba A, Shiojiri T. X-linked spinal and bulbar muscular atrophy with myasthenic symptoms. J Neurol Sci 1997;146:183–185.

36. Yang Z, Chang YJ, Yu IC, Yeh S, Wu CC, Miyamoto H, et al. ASC-J9 ameliorates spinal and bulbar muscular atrophy phenotype via degradation of androgen receptor. Nat Med 2007; 13:348–353.

Research paper

Prevalence and incidence rates of chronic inflammatory demyelinating polyneuropathy in the Japanese population

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► Supplementary tables 1–3 are published online only at http://jnnp.bmj.com/content/vol79/issue9

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Received 22 June 2007
Revised 20 December 2007
Accepted 24 December 2007
Published Online First 25 January 2008

ABSTRACT

Objective and methods: To characterise the epidemiological features of chronic inflammatory demyelinating polyneuropathy (CIDP) in the Japanese population, this study performed a nationwide assessment of the prevalence and incidence rates in Japan.

Results: The prevalence rate per 100 000 was 1.61 in the total population; 2.01 in males and 1.23 in females. The age dependent prevalence rates were 0.23 in juveniles (<15 years old), 1.50 in young adults (15–55 years) and 2.31 in elderly adults (>55 years). The sex and age dependent prevalence rates were 0.22 in males and 0.24 in females in juveniles, 1.81 in males and 1.19 in females in young adults, and 3.12 in males and 1.64 in females in elderly adults. The annual incidence rate per 100 000 was 0.48 in the total population, 0.58 in males and 0.38 in females. The age dependent incidence rate was 0.06 in juveniles, 0.40 in young adults and 0.73 in elderly adults. The sex and age dependent incidence rate was 0.05 in males and 0.08 in females in juveniles, 0.50 in males and 0.30 in females in young adults, and 0.93 in males and 0.58 in females in elderly adults. Both the prevalence and incidence rates were very similar throughout the eight geographical areas studied, from the northern to the southern parts of Japan.

Conclusions: The prevalence and incidence rates were similar to those reported in the Caucasian population. The pathogenic background is suggested to be common throughout the different races and geographic areas, while gender and age effects should be taken into account in the pathogenesis of CIDP.

Chronic inflammatory demyelinating polyneuropathy (CIDP) is a motor and sensory neuropathy with an immune mediated inflammatory element. The clinical course is divergent, taking chronic, progressive, recurrent and regressive courses; the clinical symptoms of the motor and sensory modality and its symptomatic distribution are also divergent.[1 2] The therapeutic efficacies of intravenous immunoglobulin therapy, plasma exchange and corticosteroid therapy have been established in large scale case controlled studies.[3 4] Well recognised diagnostic criteria, a thorough understanding of the pathophysiology of the disease and beneficial therapeutics have led to the acceptance of CIDP as a clinical entity.[1 5 6] However, the epidemiology of CIDP has been rarely investigated, and thus this information is lacking, particularly in Asian populations.

In this study, the prevalence and incidence rates of CIDP in the Japanese population were determined, particularly as they relate to geographical, gender and age related distributions; these data were also compared with those from Caucasian populations.

METHODS

Data for a nationwide survey were collected according to previously described methods.[7 8] As this study included paediatric and internal medicine clinics as well as neurology clinics, we collected patients with CIDP who were diagnosed by the American Academy of Neurology (AAN) criteria,[9] Saperstein's modified criteria[10] and the inflammatory neuropathy cause and treatment (INCAT) criteria.[4] We reviewed each patient's data and ascertained if the patient fulfilled these diagnostic criteria. Patients with diabetes mellitus (17.2% of collected patients), hereditary diseases and obvious paraproteinaemia were excluded from the study.

As CIDP is a chronic disease and persists in its symptoms for more than 1 year in most patients, we computed prevalence and incidence rates for 1 year of data collection.[11] We first compiled a list of all of the hospitals in Japan with 20 or more beds from data reported by the Health, Labor and Welfare Ministry in Japan. The majority of the patients with CIDP (almost 95% of the patients in the preliminary survey in the Aichi prefecture in Japan) are seen in neurology clinics of general city hospitals, or the department of neurology or department of paediatrics of university hospitals in Japan; all of these hospitals and facilities with more than 20 beds in the whole of Japan were included in this survey. A few patients with CIDP (less than 5% of the patients in the preliminary survey in the Aichi prefecture in Japan) are seen in internal medicine or paediatric clinics of the city hospitals, and thus we selected the hospitals of internal medicine and paediatrics for data sampling according to the previously described randomised selection procedure. In brief: 5% of those hospitals with 20–99 beds, 10% of those with 100–199 beds, 20% of those with 200–299 beds, 40% of those with 300–399 beds, 80% of those with 400–499 beds and 100% of the hospitals with 500 or more beds.[7 8] For departments of neurology, we selected all hospitals because we speculated that most patients with CIDP should be correctly diagnosed by neurologists. We classified all hospitals into 30 strata depending on the type of clinical department and the size of the hospital (see supplementary table 1 online). We sent questionnaires directly to

Table 1 Prevalence and incidence rates in the total Japanese population

	Male	Female	Total
Prevalence rate (/100 000)			
Juvenile 0–15 y	0.22	0.24	0.23
Adults 15+ y	2.31	1.42	1.83
Young adult 15–55 y	1.81	1.19	1.50
Elderly adult 55+ y	3.12	1.64	2.31
Total population	2.01	1.23	1.61
Incidence rate (/100 000)			
Juvenile 0–15 y	0.05	0.08	0.06
Adults 15+ y	0.67	0.43	0.54
Young adult 15–55 y	0.50	0.30	0.40
Elderly adult 55+ y	0.93	0.58	0.73
Total population	0.58	0.38	0.48

the physicians of the departments of neurology, paediatrics and internal medicine in these hospitals, and independently to each hospital, asking each for the number, gender and other clinical and experimental information of patients who were newly diagnosed as CIDP (incidence number) or had been already diagnosed as CIDP and were still receiving treatment (prevalence number) over 1 year, from the beginning of September 2004 to the end of August 2005. We also asked how they diagnosed the patients as having CIDP by referring to the diagnostic criteria of the AAN research criteria, Saperstein's modified criteria, the INCAT criteria and other diagnostic backgrounds.[10 12] In addition, we obtained information on the gender and age distribution of the Japanese population in each prefecture based on the national census (October 2005). We calculated the number of patients with CIDP in each stratum and extrapolated the prevalence and incidence figures based on the response rates to the questionnaire and the population statistics. To calculate the geographical distribution, we arranged 47 prefectures into eight areas from the north to the south of Japan and assessed the prevalence and incidence rates based on the population in each area.

This study was performed as a project study in the Refractory Peripheral Neuropathy Research Study Group, under the auspices of the Ministry of Health, Labor and Welfare of Japan. The study design was agreed upon and approved by the Ethics Committee of Nagoya University Graduate School of Medicine.

RESULTS

The study received 1561 responses to the questionnaire out of 2827 surveyed facilities, for a total net recovery rate of 55.2%; 51.8% of the neurology clinics, 41.8% of the clinics of internal medicine and 70.1% of the paediatric clinics (see supplementary table 1 online). From September 2004 to August 2005, 742 men and 480 women were diagnosed with CIDP in the 1561 medical facilities out of the total of 2827 randomly selected surveyed hospitals in Japan having more than 20 beds. Based on these data, and the response rates from each stratum of the facilities, we obtained a prevalence number of 2433 patients (1495 men and 938 women) (see supplementary table 2 online). The CIDP prevalence rate per 100 000 of the Japanese population was 1.61 in the total population, 2.01 in the male population and 1.23 in the female population (table 1). The age dependent prevalence rate was 0.23 in juveniles, 1.50 in young adults and 2.31 in elderly adults. The sex dependent prevalence rate in each age group was 0.22 in males and 0.24 in females in juveniles, 1.81 in males and 1.19 in females in young adults, and 3.12 in males and 1.64 in females in elderly adults. The number of newly diagnosed patients with CIDP during the year from September 2004 to August 2005 was 601 (354 men and 247 women) (see supplementary table 3 online). The annual incidence rate per 100 000 was 0.48 in the total population, 0.58 in males and 0.38 in females. The age dependent annual incidence rate was 0.06 in juveniles, 0.40 in young adults and 0.73 in elderly adults. The sex dependent incidence rate in each group was 0.05 in males and 0.08 in females in juveniles, 0.50 in males and 0.30 in females in young adults, and 0.93 in males and 0.58 in females in elderly adults (table 1).

Additionally, there was no difference in the prevalence or the incidence rates in the total population in eight geographical areas (Hokkaido, Tohoku, Kanto, Koshin-etsu, Tokai, Kinki, Chugoku-Shikoku and Kyushu-Okinawa) in Japan (fig 1).

DISCUSSION

The higher prevalence and incidence rates in males compared with females, and the increasing rates with aging were the

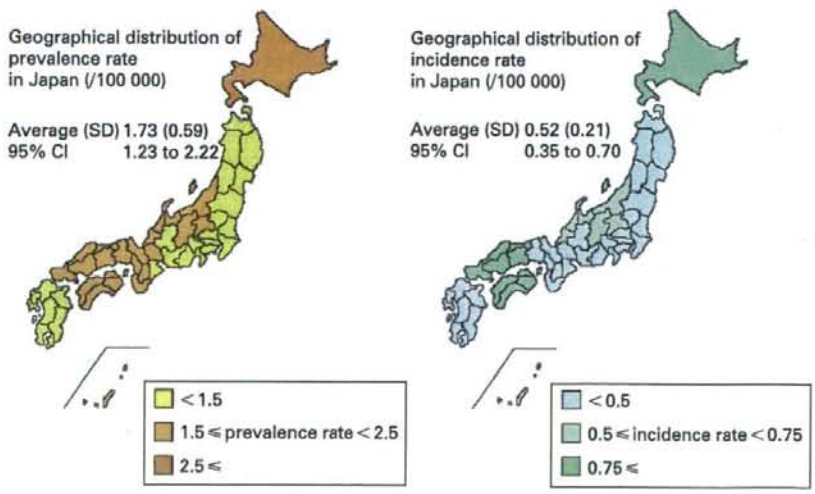

Figure 1 Geographic distribution of prevalence and incidence rates throughout Japan. There were no statistical preponderances in the geographical distributions of either the prevalence or incidence rates.

Table 2 Comparison of the prevalence and incidence rates among Caucasian and Japanese populations

Report	Location	Population	Prevalence rate (/100 000)			Incidence rate (/100 000)		
			Total	Adult	Juvenile	Total	Adult	Juvenile
Lunn[13] (1994–1995)	England (UK)	14 049 000	0.46–1.24	NA*	NA*	NA	NA	NA
McLeod[14] (1996)	New South Wales (Australia)	5 995 000	1.87	NA	0.48†	NA	NA	NA
	Newcastle (Australia)	448 000	NA	NA	NA	0.15	NA	NA
Chio[15] (2001)	Piemonte and Valle d'Aosta (Italy)	4 334 225	3.58	NA*	NA*	0.36	NA	NA
Iijima (2004–2005)	Whole areas of Japan (Japan)	127 655 000	1.61	1.83	0.23‡	0.48	0.54	0.06‡

*Although the exact data were not reported, the age dependent increase in the prevalence rate was discussed in each report.
†Juvenile population is designated as those under 20 years.
‡Juvenile population is designated as those under 15 years.
NA, not available.

major observations of the Japanese epidemiology of CIDP, as was the lack of a specific geographical distribution. As CIDP is a chronic disease generally lasting more than 1 year, our results on observations over 1 year are expected to represent the transverse epidemiology in the Japanese population.

A few well designed epidemiological studies have been reported from the UK, Australia and the north of Italy in Caucasian populations.[13–15] The most striking finding was that our data in the Japanese population were similar to those reported in these Caucasian populations (table 2). Compared with the UK–Australian data, we found epidemiological similarity in the total prevalence and incidence rates, male predominance over females, and the higher prevalence and incidence rates in the adult population compared with the juvenile population, although the ages categorising their juvenile populations were different to ours. The prevalence rate in northern Italy was slightly higher than ours (table 2), while the increasing prevalence and incidence rates in their elderly populations were similar to ours. The prevalence and incidence rates in our study may be somewhat underestimated as we excluded patients with diabetes mellitus or paraproteinaemia; these data were also collected in a hospital based manner, excluding those under home care or under private office follow-up not attending hospital during the survey period. Another source of bias is that we would have missed patients who were diagnosed before the survey, but who did not attend hospital during the survey period, which may have occurred because their disease was too mild, or patients were too ill or did not see any point in attending because their treatment was not helping.

In addition, our results clearly demonstrate that there is no significant preponderance in the geographical distribution from the north to the south of Japan for the epidemiology of CIDP. These results suggest that CIDP is similar in its epidemiological background in different races and different geographical environments, indicating that the pathogenesis of CIDP could be common worldwide, and independent of genetic and geographical environmental influences, although further studies are needed to confirm this.

Another interesting observation was the gender related difference in the prevalence and incidence rates. In the adult population, prevalence and incidence rates were significantly higher in males; the male to female ratio was 1.63 to 1 (1.52 to 1 in young adults and 1.90 to 1 in elderly adults) for the prevalence rate and 1.56 to 1 (1.67 to 1 in young adults and 1.60 to 1 in elderly adults) for the incidence rate. Whereas in the juvenile population a significant preponderance was observed in girls, the male to female ratio was 0.92 to 1 for the prevalence rate and 0.63 to 1 for the incidence rate. At present we do not understand the background mechanism underlying this gender related difference, particularly its reversed ratio among the adult and juvenile populations.[11 16] However, the gender and age related differences in the epidemiological indices were remarkable, especially given their reversal during puberty, suggesting that the effects of gender could be significant in the pathogenesis of CIDP.

Funding: This study was supported by the grants from the Ministry of Health, Labor and Welfare of Japan.

Competing interests: None.

Ethics approval: The study design was agreed upon and approved by the Ethics Committee of Nagoya University Graduate School of Medicine.

REFERENCES

1. **Dyck PJ,** O'Brien PC, Oviatt KF, *et al*. Prednisone improves chronic inflammatory demyelinating polyradiculoneuropathy more than no treatment. *Ann Neurol* 1982;**11**:136–41.
2. **Barohn RJ,** Kissel JT, Warmolts JR, *et al*. Chronic inflammatory demyelinating polyradiculoneuropathy. Clinical characteristics, course, and recommendations for diagnostic criteria. *Arch Neurol* 1989;**46**:878–84.
3. **Hahn AF,** Bolton CF, Pillay N, *et al*. Plasma-exchange therapy in chronic inflammatory demyelinating polyneuropathy. A double-blind, sham-controlled, cross-over study. *Brain* 1996;**119**(Pt 4):1055–66.
4. **Hughes R,** Bensa S, Willison H, *et al*. Randomized controlled trial of intravenous immunoglobulin versus oral prednisolone in chronic inflammatory demyelinating polyradiculoneuropathy. *Ann Neurol* 2001;**50**:195–201.
5. **McCombe PA,** Pollard JD, McLeod JG. Chronic inflammatory demyelinating polyradiculoneuropathy. A clinical and electrophysiological study of 92 cases. *Brain* 1987;**110**(Pt 6):1617–30.
6. **Hughes RA,** Allen D, Makowska A, *et al*. Pathogenesis of chronic inflammatory demyelinating polyradiculoneuropathy. *J Peripher Nerv Syst* 2006;**11**:30–46.
7. **Kojima M,** Wakai K, Tokudome S, *et al*. Serum levels of polyunsaturated fatty acids and risk of colorectal cancer: a prospective study. *Am J Epidemiol* 2005;**161**:462–71.
8. **Nakamura Y,** Matsumoto T, Tamakoshi A, *et al*. Prevalence of idiopathic hypoparathyroidism and pseudohypoparathyroidism in Japan. *J Epidemiol* 2000;**10**:29–33.
9. **Research criteria for diagnosis of chronic inflammatory demyelinating polyneuropathy (CIDP).** Report from an Ad Hoc Subcommittee of the American Academy of Neurology AIDS Task Force. *Neurology* 1991;**41**:617–18.
10. **Saperstein DS,** Katz JS, Amato AA, *et al*. Clinical spectrum of chronic acquired demyelinating polyneuropathies. *Muscle Nerve* 2001;**24**:311–24.
11. **Iijima M,** Yamamoto M, Hirayama M, *et al*. Clinical and electrophysiologic correlates of IVIg responsiveness in CIDP. *Neurology* 2005;**64**:1471–5.
12. **Sander HW,** Latov N. Research criteria for defining patients with CIDP. *Neurology* 2003;**60**:S8–15.
13. **Chio A,** Cocito D, Bottacchi E, *et al*. Idiopathic chronic inflammatory demyelinating polyneuropathy: an epidemiologic study in Italy. *J Neurol Neurosurg Psychiatry* 2007;**78**:1349–53.
14. **McLeod JG,** Pollard JD, Macaskill P, *et al*. Prevalence of chronic inflammatory demyelinating polyneuropathy in New South Wales, Australia. *Ann Neurol* 1999;**46**:910–13.

15. **Lunn MP,** Manji H, Choudhary PP, *et al*. Chronic inflammatory demyelinating polyradiculoneuropathy: a prevalence study in south east England. *J Neurol Neurosurg Psychiatry* 1999;**66**:677–80.
16. **McCombe PA,** Csurhes PA, Greer JM. Studies of HLA associations in male and female patients with Guillain–Barre syndrome (GBS) and chronic inflammatory demyelinating polyradiculoneuropathy (CIDP). *J Neuroimmunol* 2006;**180**:172–7.

APPENDIX

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Molecular Genetics and Biomarkers of Polyglutamine Diseases

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Abstract: Polyglutamine diseases are hereditary neurodegenerative disorders caused by an abnormal expansion of a trinucleotide CAG repeat, which encodes a polyglutamine tract. To date, nine polyglutamine diseases are known: Huntington's disease (HD), spinal and bulbar muscular atrophy (SBMA), dentatorubral-pallidoluysian atrophy (DRPLA) and six forms of spinocerebellar ataxia (SCA). The diseases are inherited in an autosomal dominant fashion except for SBMA, which shows an X-linked pattern of inheritance. Although the causative gene varies with each disorder, polyglutamine diseases share salient genetic features as well as molecular pathogenesis. CAG repeat size correlates well with the age of onset in each disease, shows both somatic and germline instability, and has a strong tendency to further expand in successive generations. Aggregation of the mutant protein followed by the disruption of cellular functions, such as transcription and axonal transport, has been implicated in the etiology of neurodegeneration in polyglutamine diseases. Although animal studies have provided promising therapeutic strategies for polyglutamine diseases, it remains difficult to translate these disease-modifying therapies to the clinic. To optimize "proof of concept", the process for testing candidate therapies in humans, it is of importance to identify biomarkers which can be used as surrogate endpoints in clinical trials for polyglutamine diseases.

Keywords: Polyglutamine, Huntington's disease, spinal and bulbar muscular atrophy, spinocerebellar ataxia, dentatorubral-pallidoluysian atrophy, biomarker, surrogate endpoint.

INTRODUCTION

Neurodegenerative diseases are a group of devastating disorders which enfeeble movement and/or cognitive functions by affecting a certain population of neurons within the central nervous system. The complexity in the pathogenesis of neurodegeneration in these disorders has resulted in few available and effective therapies. Although the disease entities were mostly described in the late nineteenth century, it was recent advances in molecular biology that identified the causative gene mutations in familial cases and thereby dramatically expanded the understanding of the pathogenesis of neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease and polyglutamine diseases. Continual efforts to understand the disease mechanisms have identified the accumulation of intracellular and extracellular proteins as the molecular basis for various neurodegenerative diseases. Moreover, the creation of animal models that recapitulate human pathology has been driving the translation of biological insights into disease-modifying therapies which inhibit the core events of the pathogenesis in neurodegeneration. These therapeutic approaches, however, are now challenged by drawbacks in clinical trials for neurodegenerative diseases: limited pools of potential participants, insensitive outcome measures and the slow progression of disease. To circumvent these obstacles, it is desirable to identify biomarkers with which to monitor disease progression and responses to therapies. In this review, several attempts to identify biomarkers for polyglutamine diseases and hereditary neurodegenerative disorders caused by unique gene mutations will be discussed.

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1566-5240/08 $55.00+.00

CLINICAL AND GENETICAL FEATURES OF POLYGLUTAMINE DISEASE

Epidemiology

To date, nine polyglutamine diseases are known: Huntington's disease (HD); spinal and bulbar muscular atrophy (SBMA); dentatorubral-pallidoluysian atrophy (DRPLA) and six forms of spinocerebellar ataxia (Table (**1**)). The best characterized polyglutamine disease is HD, which affects 5-7 individuals out of 100,000 in western countries. Although HD is unusually common in certain areas including Tasmania and the villages around Lake Maracaibo in Venezuela, the prevalence of this disease is not more than 1 per 100,000 in Asia [1]. SBMA, also known as Kennedy's disease, exclusively affects males with a prevalence of 1-2 per 100,000 in the total population [2]. The prevalence of DRPLA is high in the Japanese population, 0.2-0.7 in 100,000, but patients have also been identified in other populations including Europe and North America [3]. Haw River syndrome, an autosomal dominant disorder found

Table 1. Classification of Polyglutamine Diseases

Disease	Major Clinical Features	Affected Regions	Causative Protein	Gene (Locus)
Huntington's disease (HD)	Chorea, cognitive deficits, psychiatric disturbances	Striatum, cerebral cortex	Huntingtin	*IT15* (4p16.3)
Spinal and bulbar muscular atrophy (SBMA, Kennedy disease)	Weakness, muscular atrophy, bulbar palsy	Spinal cord, brainstem	Androgen receptor	*AR* (Xq13-q12)
Spinocerebellar ataxia type 1 (SCA1)	Ataxia, bulbar palsy, pyramidal signs, muscular atrophy	Cerebellum, brainstem	Ataxin 1	*SCA1* (6p23)
Spinocerebellar ataxia type 2 (SCA2)	Ataxia, slow eye movement, neuropathy	Cerebellum, brainstem	Ataxin 2	*SCA2* (12q24.1)
Spinocerebellar ataxia type 3 (SCA3, Machado-Joseph disease)	Ataxia, bulging eye, parkinsonism, spasticity, fasciculations	Cerebellum, basal ganglia, brainstem, spinal cord	Ataxin 3	*SCA3/MJD* (14q32.1)
Spinocerebellar ataxia type 6 (SCA6)	Ataxia	Cerebellum	α1A-voltage-dependent calcium channel subunit	*CACNA1A* (19p13)
Spinocerebellar ataxia type 7 (SCA7)	Ataxia, retinal degeneration	Cerebellum, retina, brainstem, visual cortex	Ataxin 7	*SCA7* (3p12-p13)
Spinocerebellar ataxia type 17 (SCA17)	Ataxia, cognitive deficits, dystonia, parkinsonism	Cerebellum, striatum	TATA box binding protein	*TBP* (6q27)
Dentatorubral-pallidoluysian atrophy (DRPLA)	Ataxia, myoclonic epilepsy, choreoathetosis, cognitive deficits	Cerebellum, cerebral cortex, globus pallidus, red nuclei, subthalamic nuclei	Atrophin 1	*DRPLA* (12p13.31)

in African American families in North Carolina, is genetically identical to DRPLA [4,5]. The prevalence of polyglutamine-associated SCAs, among which SCA3 is the most common, has been estimated to be not more than 3-5 per 100,000.

Symptoms

In general, symptoms of polyglutamine diseases typically appear in mid-life and progressively deteriorate before death from fatal complications. Clinical features vary for each disorder, corresponding to the pathological distribution of neurodegeneration.

Individuals with HD can become symptomatic at any time between the ages of one and 80 years, with the mean at 35 to 44 years. HD is characterized by movement disorder, early cognitive signs and psychiatric disturbances. Chorea is the most common motor symptom in adult HD cases, whereas parkinsonism may be predominant in juvenile-onset patients. Cognitive dysfunction in HD impairs attention, execution and memory, and shows a stepwise progression over time. Depression and suicidal ideation are also frequent in HD patients. Another clinical finding in HD is motor impersistence, the inability to sustain a voluntary muscle contraction such as tongue protrusion. After progressive deterioration for 10-20 years, HD patients die from complications of falls, inanition, dysphagia or aspiration.

Major symptoms of SBMA are weakness, atrophy and fasciculations of bulbar, facial and limb muscles [6]. Patients with SBMA occasionally demonstrate signs of androgen insensitivity such as gynecomastia, testicular atrophy, impaired erection and decreased fertility, some of which are detected before the onset of motor impairment. Female carriers are usually asymptomatic, but some express subclinical phenotypes including high amplitude motor unit potentials on electromyography. The progression of SBMA is usually slow, but life-threatening respiratory tract infection often occurs in the advanced stages of the disease, resulting in early death in some patients. The cardinal cause of death is aspiration pneumonia [7].

Symptoms of DRPLA include progressive ataxia, epilepsy, myoclonus, choreoathetosis and intellectual decline, a variable combination of which is observed in each patient. Progressive myoclonic epilepsy characterized by myoclonus, seizures, ataxia and progressive intellectual deterioration is predominant in early-onset cases, most of whom inherit their disease alleles from their affected fathers. In contrast, choreoathetosis and cerebellar ataxia are major symptoms in late-onset patients of DRPLA [8]. Some patients with DRPLA may

also exhibit psychosis in addition to extrapyramidal and cerebellar symptoms. The age of onset is from 1 to 62 years with the mean at 30 years.

The main symptoms of SCAs are cerebellar ataxia and dysarthria, although additional signs might also be observed. SCA1 patients may present with pyramidal signs, muscular atrophy and peripheral neuropathy, whereas SCA2 is characterized by slow eye movement and hypo- or areflexia. Bulging eye, parkinsonism, spasticity and facial and lingual fasciculations might suggest SCA3. SCA6 is typified by pure cerebellar dysfunction such as ataxia, dysarthria and nystagmus, whereas visual impairment due to retinal degeneration is often observed together with ataxia in SCA7 patients [9]. Cognitive decline, dystonia and parkinsonism characterize the clinical presentation of SCA17 [10]. Although symptomatology may help distinguish each SCA, clinical diagnosis is hampered by variability in neurological phenotypes. Gene analysis is thus indispensable for the diagnosis of polyglutamine-induced SCAs.

Genetics

Polyglutamine diseases are hereditary neurodegenerative disorders caused by an abnormal expansion of a trinucleotide CAG repeat, which encodes a polyglutamine tract [11]. The CAG repeat length in the causative genes shows virtually no overlap between unaffected individuals and the patients with polyglutamine diseases. The diseases are inherited in an autosomal dominant fashion except for SBMA, which occurs only in males because of testosterone-dependency in the pathogenesis of the disease [12]. The pathogenic threshold for the disease is approximately 35-40 CAGs in all polyglutamine-mediated disorders except for SCA6, in which disease develops when the expansion exceeds 21 CAGs.

Although causative gene products are unrelated outside of the polyglutamine stretch, these disorders share salient genetic features. For example, CAG repeat size correlates well with the age of onset in each disease: patients with a longer CAG repeat tend to demonstrate an earlier onset of symptoms. The size of the CAG repeat, at least partially, accounts for the differences between the clinical features of early-onset juvenile cases and those in late-onset adult patients. Besides, CAG repeats show both somatic and germline instability, and have a strong tendency to further expand in successive generations, leading to the phenomenon of anticipation, in which subsequent generations have an earlier onset and more severe symptoms. The strong correlation between CAG repeat size and the age of onset is the basis for the estimation of disease onset in preclinical mutation carriers of HD [13].

Histopathology

In each polyglutamine disease, there is loss of a specific subset of neurons despite the widespread expression of the causative gene products throughout the central nervous system (Table (**1**)). Regional degeneration may result in the atrophy of neuronal tissues which is visible in computed tomography (CT) or magnetic resonance imaging (MRI) scans. For example, HD is characterized by the atrophy of the caudate and putamen together with an enlargement of the frontal horns of the lateral ventricles. Brain-imaging sc Bates ns demonstrate cerebellar atrophy with or without the loss of pontine volume in most SCA cases and often reveal cerebral and cerebellar atrophy in DRPLA patients. The abnormal polyglutamine proteins are also expressed outside the nervous system, leading to non-neuronal pathology, such as diabetes mellitus, in some polyglutamine diseases [14,15].

Although the population of vulnerable cells varies for the diseases, the abnormal polyglutamine protein forms inclusion bodies in affected neurons, which is a unifying histopathological hallmark of polyglutamine diseases [16]. These neuronal inclusion bodies are often detected in the nucleus, although they may be formed within the cytoplasm or neurites. The deposition of inclusion bodies is not only found in the postmortem neural tissues from patients, but has also been reported in animal models of polyglutamine diseases. The abnormal polyglutamine proteins in the inclusion bodies are often truncated, indicating that proteolytic cleavage appears to enhance the toxicity of the causative gene products [17]. Many components of the ubiquitin-proteasome pathway and molecular chaperones colocalize with inclusion bodies, implying that a failure of cellular defense mechanisms underlies neurodegeneration in polyglutamine diseases. Although the formation of neuronal inclusion bodies is a disease-specific histopathological finding, its role in the pathogenesis of disease has been heavily debated. Several studies have suggested that polyglutamine inclusion bodies may indicate a cellular response coping with the toxicity of abnormal polyglutamine proteins [18]. Instead, in SBMA and DRPLA, the diffuse nuclear accumulation of the abnormal polyglutamine proteins has been considered to be essential for inducing neurodegeneration (Fig. (**1**)) [19,20].

MOLECULAR PATHOGENESIS AND THERAPEUTIC DEVELOPMENT

Pathogenesis

The expanded polyglutamine tract in the causative gene product has been implicated in the pathogenesis of polyglutamine diseases in two different, but not mutually exclusive, ways: 1) loss of normal function of the protein induces neuronal degeneration; and 2) the causative protein acquires toxic properties that damages neurons. Although the expansion of the polyglutamine tract may suppress the normal function of the protein, the removal of the causative protein genetically or by other means fails to result in disease in either humans or animal models. Therefore, the main cause of neurodegeneration in polyglutamine diseases appears to be

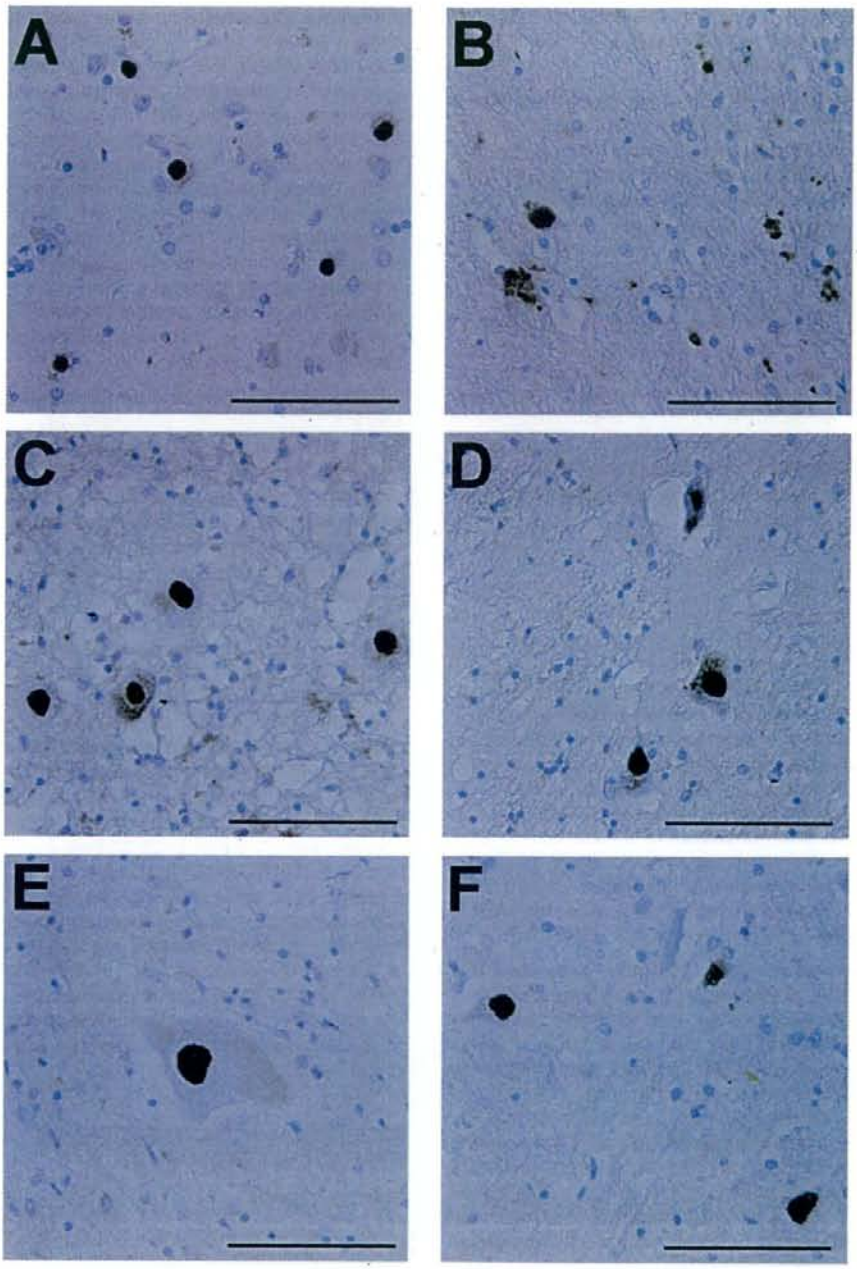

Fig. (1). Accumulation of abnormal proteins in polyglutamine diseases. Immunohistochemistry of autopsy specimens from patients using an anti-polyglutamine antibody (1C2). A. Cerebral cortex, HD; B. Putamen, HD; C. Dentate nucleus, DRPLA, D. globus pallidus, DRPLA; E. Anterior horn of spinal cord, SBMA; F. Pons, SBMA. Scale bar = 100 μm.

a gain of toxic function of the causative protein containing the expanded polyglutamine tract.

Accumulation of abnormal proteins has been considered to be central to the pathogenesis of neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis (ALS) and prion disease [21]. It is now widely accepted that the accumulation of causative proteins in neurons is an important event in the pathogenesis of polyglutamine diseases. Proteins with expanded polyglutamine seg-

ments have an altered conformation, resulting in aggregation of the proteins. These polyglutamine aggregates are formed in neurons, when the production of causative proteins overwhelms the cellular protective capacity, such as the ubiquitin-proteasome system and molecular chaperones. The propensity to aggregate of aberrant proteins is dependent on the polyglutamine stretch length. The protein accumulation probably underlies some clinical features of polyglutamine diseases, such as the late onset of symptoms, progressive disease course and the dependence of onset age on polyglutamine length [22]. Several experimental observations indicate that the formation of toxic oligomers, or intermediates, of abnormal polyglutamine-containing proteins instigates a series of cellular events which lead to neurodegeneration [23]. Although the aggregated polyglutamine proteins also form inclusion bodies, these amyloid-like fibrils might be less toxic than oligomers. On the other hand, recent studies have suggested that soluble β-sheet monomers of abnormal polyglutamine protein appear to be toxic [24].

Disruption of the transcriptional machinery has also been hypothesized to underlie the pathogenesis of polyglutamine diseases [25]. Transcriptional co-activators such as CREB-binding protein (CBP) are sequestrated into the polyglutamine-containing inclusion bodies through protein-protein interactions in mouse models and in patients with polyglutamine diseases [26]. Alternatively, the interaction between transcriptional co-activators and soluble pathogenic proteins has also been demonstrated in animal models of polyglutamine diseases as well as in postmortem tissues of patients [27]. CBP functions as a histone acetyltransferase (HAT), regulating gene transcription and chromatin structure. It has been indicated that the HAT activity of CBP is suppressed in cellular models of polyglutamine diseases.

Several lines of evidence suggest that oxidative stress and mitochondrial dysfunction are implicated in the pathogenesis of polyglutamine diseases. Mitochondrial defects have been shown in the brains of HD mice as well as in lymphoblasts from HD patients [28]. Impaired activities of oxidative phosphorylation enzymes have also been observed in the postmortem HD striatum [29]. The levels of deletions in mitochondrial DNA, another indicator of oxidative stress, are increased in the cerebral cortex of HD patients [30]. Oxidative damage may also result from transcriptional dysregulation of peroxisome proliferators-activated receptor-γ coactivator 1α, which regulates the expression of various mitochondrial proteins [31].

Apoptotic pathway has also been proposed to play an important role in the pathogenesis of polyglutamine diseases. A number of cell-based studies have shown that the expression of abnormal polyglutamine protein activates apoptotic pathway and induces apoptosis. Pro-apoptotic caspases, such as caspase-1 and -8, are activated in the brain of HD patients, and both genetic and pharmacological inhibition of caspase slows disease progression in a mouse model of HD [32,33]. Calpain, another mediator of apoptosis, has also been shown to be activated in HD brain [34]. Furthermore, it should be noted that caspases and calpain enhance the toxicity of aberrant proteins through protein cleavage.

Abnormal polyglutamine proteins have also been shown to inhibit anterograde and/or retrograde axonal transport, suggesting that disrupted axonal transport appears to underlie the pathogenesis of HD and SBMA. [35,36]. Alternatively, it has been demonstrated that polyglutamine-dependent transcriptional dysregulation of dynactin 1, an axon motor regulating retrograde transport protein, plays a crucial role in the reversible neuronal dysfunction observed in the early stages of SBMA [37]. The pathogenic androgen receptor (AR) containing expanded polyglutamine, the causative protein of SBMA, has also been demonstrated to activate c-Jun N-terminal kinase (JNK), leading to an inhibition of kinesin-1 microtubule-binding activity and the eventual disruption of anterograde axonal transport [38].

In mouse models of polyglutamine diseases, it has been postulated that neuronal dysfunction, without cell loss, is sufficient to cause neurological symptoms [39]. These observations indicate that the pathogenesis of polyglutamine diseases is potentially reversible at early stages (Fig. (**2**)). Indeed, arrest of gene expression after the onset of symptoms reverses the behavioral and neuropathological abnormalities in conditional mouse models of polyglutamine diseases [40,41]. This hypothesis is also supported by the fact that the neuronal cell death is often undetectable in mildly affected HD patients despite the presence of definite clinical features [42].

Therapeutic Strategies

There is no disease-modifying therapy which has been proven to be effective in clinical trials, although some drugs have been used for symptom relief, such as tetrabenezine or neuroleptics for chorea in HD. Potential therapeutics, however, have emerged from basic research using animal models of polyglutamine diseases.

Because the accumulation of aberrant proteins is an important target of therapeutics in polyglutamine diseases, several efforts have been made to inhibit aggregate formation in cellular and animal models. High-throughput screening systems have been employed in an attempt to identify compounds which inhibit the accumulation of abnormal polyglutamine proteins [43]. Oral administration of trehalose, the most effective disaccharide identified in an *in vitro* aggregate formation assay, suppressed polyglutamine accumulation in the brain, improved motor dysfunction and extended life span in a transgenic mouse model of HD [44]. In SBMA, anti-polyglutamine therapies have been developed taking advantage of the fact that the accumulation of the pathogenic AR proteins is dependent on the circulating level of testosterone [45,46]. It should be noted that surgical castration reverses motor dysfunc-

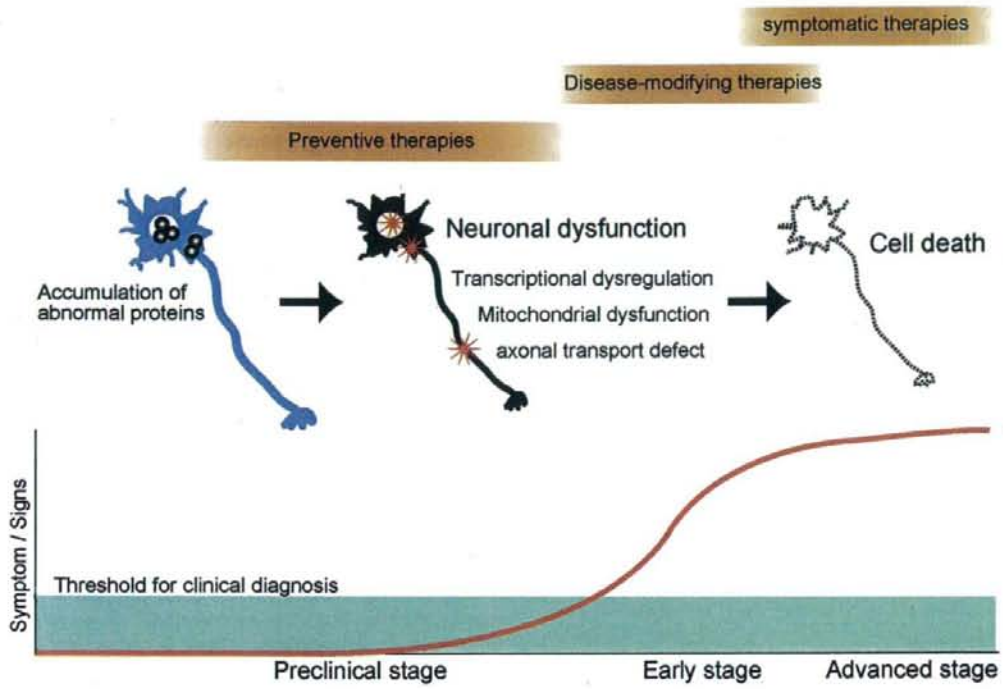

Fig. (2). Pathogenesis of polyglutamine diseases and the role of biomarkers. Accumulation of causative proteins, the culprit of neurodegeneration, triggers several downstream events in the pathogenesis of polyglutamine diseases: dysregulation of transcription, mitochondrial dysfunction and disruption of axonal transport. These events result in neuronal dysfunction, which has been considered to be partially reversible in the early stages of the disease. Biomarkers might be used for the selection of patients who are likely to benefit from interventions, and for the monitoring of responses to the preventive, disease-modifying and symptomatic therapies.

tion in mouse models of SBMA [47]. The luteinizing hormone-releasing hormone analogue, leuprorelin, prevents nuclear translocation of aberrant AR proteins, resulting in a significant improvement of disease phenotype in a mouse model of SBMA [48].

Activation of the cellular defense machinery is another promising therapeutic approach for polyglutamine diseases. Over-expression of heat shock proteins (HSPs), stress-inducible molecular chaperones, inhibits toxic accumulation of abnormal polyglutamine protein and suppresses neurodegeneration in a variety of cellular and animal models of polyglutamine diseases [49-52]. Similar beneficial effects have also been achieved by the pharmacological induction of HSPs [53,54]. On the other hand, inhibition of Hsp90 has been demonstrated to arrest neurodegeneration by activating the ubiquitin-proteasome system in polyglutamine diseases. Treatment with 17-allylamino geldanamycin (17-AAG), a potent Hsp90 inhibitor, dissociated p23 from the Hsp90-AR complex, and thus facilitated proteasomal degradation of the pathogenic AR in cellular and mouse models of SBMA [55,56]. Autophagy has also been attracting attention as another clearance mechanism of abnormal polyglutamine proteins. Rapamycin, an inducer of autophagy, improved behavioral abnormalities and decreased aggregate formation in a mouse model of HD [57].

Transcriptional dysregulation is another target for therapeutic intervention. Because suppression of histone deacetylase (HDAC) activities results in an augmentation of histone acetylation and a subsequent restoration of gene transcription, HDAC inhibitors have been considered to be of therapeutic benefit in polyglutamine diseases [27]. Butyrate was the first HDAC inhibitor to be discovered and the related compound, phenylbutyrate, has been successfully employed in experimental cancer therapy. Oral administration of sodium butyrate ameliorates the symptomatic and histopathological phenotypes of a mouse model of SBMA through upregulation of histone acetylation in nervous tissues [58]. This compound has also been shown to alleviate neurodegeneration in a mouse model of DRPLA [59]. In mouse models of HD, the administration of HDAC inhibitors: sodium butyrate; suberoylanilide hydroxamic acid and phenylbutyrate; has been shown to alleviate polyglutamine toxicity and improve neuronal dysfunction [60-62].

Other potential therapeutic compounds for polyglutamine diseases include: antioxidant agents (e.g. creatinine, coenzyme Q10, remacemide and lipoic acid);

caspase inhibitors (e.g. minocycline); transglutaminase inhibitors (e.g. cysteamine); anti-apoptotic agents (e.g. tauroursodeoxycholic acid); excitotoxicity blockers (e.g. amantadine and memantine) and lithium [63]. In addition to pharmacological approaches, RNA interference, peptide inhibitors, cell transplantation and trophic factor supplementation are all potential therapeutic strategies, if safety and delivery problems are solved.

BIOMARKERS FOR POLYGLUTAMINE DISEASE

The Need for Biomarkers of Polyglutamine Diseases

A biomarker is defined as "a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes or pharmacologic responses to a therapeutic intervention" [64]. Biomarkers are thus very important tools to distinguish presymptomatic or mildly affected patients from unaffected individuals, to monitor disease progression and to determine the efficacy of therapeutics in clinical trials. The use of biomarkers effectuates several steps in therapeutic research: the selection of lead compounds for confirmative clinical trials; the designing of interventional studies; understanding the pharmacological profiles of candidate compounds; distinction of individuals who are likely to benefit from therapies and those who are susceptible to adverse effects (Fig. (**2**)). Some biomarkers may also be employed as surrogate endpoints, the parameters intended to substitute clinical endpoints in interventional trials. Not only do these markers serve to accelerate the development of therapies, but also build a bridge from the animal models to the human disease by identifying common molecular events shared by animals and patients.

The disease progression of neurodegenerative disorders including polyglutamine diseases is fairly slow and highly variable, hampering the sensitive detection of therapeutic effects in clinical trials. For example, the Total Functional Capacity Scale (TFC), a 14-unit (range 0-13) functional scale for HD, declined 0.72 units per year in a large cohort study, suggesting that more than 700 patients would be needed to conduct a 2-year clinical trial [65]. Clinical scales such as TFC are also subject to some degree of inter- and intra-rater variability. Because the number of patients in each polyglutamine disease is small, trials targeting clinical endpoints would also require long observation periods, which might diminish the motivation of participants. The use of biomarkers may reduce sample sizes and shorten trial duration and thus is expected to facilitate "proof of concept", the process for testing candidate therapies in exploratory clinical studies, for polyglutamine diseases. In addition, biomarker measurement has been demonstrated to track disease progression in at-risk mutation carriers of HD showing no definitive clinical phenotypes [13].

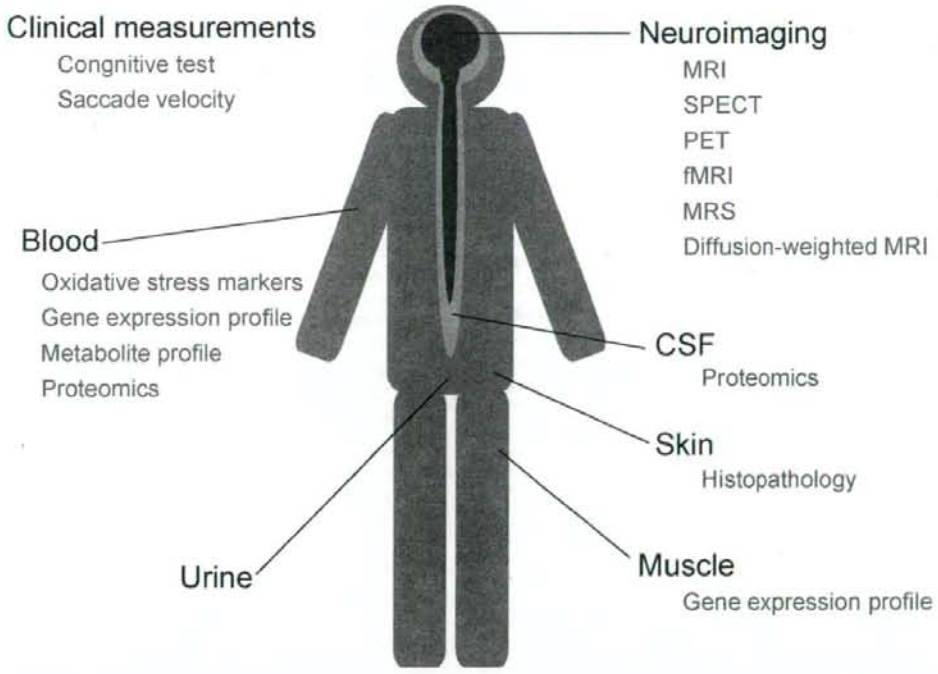

Fig. (3). Potential biomarkers for polyglutamine diseases. Animal and clinical studies have provided candidate biomarkers for polyglutamine diseases, including: neuroimaging; biofluid analysis; clinical measurement; gene expression profiles; proteomics; metabonomic analysis and histopathology; some of which have been tested in preliminary clinical trials.

Theoretically, biomarkers for neurodegenerative diseases should detect disease-specific neuropathology, non-specific neurodegenerative processes or biological events downstream of neuronal dysfunction. Ideal biomarkers are replicable, non-invasive, reliable, precise and inexpensive variables which are easy to measure. Currently studied biomarkers for polyglutamine diseases include: parameters of neuroimaging; biofluid analysis; physical assessment; gene expression profiles; proteomics; metabonomic analysis and histopathology, some of which have been investigated in preliminary clinical trials (Fig. (**3**)) [66].

Neuroimaging Markers

Because regional brain atrophy is a fundamental neuropathological finding in HD and SCAs, neuroimaging parameters have been the most popular biomarkers for polyglutamine diseases. In addition to assessment of brain structures, imaging techniques also provide important information on function, metabolites or perfusion. Recent advances in molecular imaging would enable the detection of causative protein accumulation and disease-specific molecular pathogenesis [67,68].

1. Structural Neuroimaging

Structural imaging studies using CT or MRI have delineated the correlation between brain morphology and cognitive functions in HD patients. Several neuroradiological studies have shown that the earliest changes in HD appear in the striatum (caudate, putamen and globus pallidus), the degree of which has been intensively studied in quantitative studies using MRI. The caudate volumes have been reported to correlate with the Mini Mental State Examination (MMSE) and with performances on neurophysiological tests [69,70]. The volumes of the caudate and putamen are significantly decreased in preclinical HD carriers who are far from their estimated age of disease onset as calculated from CAG size, and the rate of caudate atrophy becomes significant approximately 11 years from the estimated age of onset [71]. In addition, more generalized brain atrophy has also been detected with MRI, suggesting that the rate of whole brain atrophy is also a potential biomarker [72,73]. Recently, computational neuroanatomic techniques have been applied to neuroimaging of HD. Voxel-based morphometry (VBM), a fully automated method that detects regional structural changes on a voxel-wise basis, delineates reduction in gray matter volume in the striatum of presymptomatic HD carriers [74]. Tensor-based morphometry, another automated technique, also demonstrates progressive regional gray matter atrophy within the striatum of preclinical at-risk HD carriers [75].

Regional atrophy has also been reported in polyglutamine-mediated SCAs. MRI volumetry using manual segmentation has also detected a significant atrophy of the cerebellum and brainstem in SCA1, SCA2, SCA3 and SCA7 patients [76-80]. The brainstem volume in MRI correlates with the Inherited Ataxia Clinical Rating Scales (ICARS) in SCA1 but not in SCA2 [80]. VBM has also been applied to patients with SCAs, but it has yet to be defined whether parameters in this method can be used as biomarkers [81,82].

2. Functional Imaging of Resting State

Functional neuroimaging is intended to measure regional cerebral blood flow (rCBF), metabolites or neurotransmission activity as indicators of brain functions. There are two major classes of functional imaging methods: one examines the resting-state activity and the other measures the changes in brain activity during the performance of a task. In HD, neuroimaging studies of resting cerebral metabolism using positron emission tomography (PET) and single-photon emission computed tomography (SPECT) have demonstrated metabolic abnormalities in the striatal and extra-striatal regions. Reduced rCBF in frontotemporal regions and in the caudate have been reported to correlate with cognitive functions [83,84]. Glucose hypometabolism has also been revealed in PET, the degree of which correlates with cognitive deficits in HD patients [85,86] Reduction in glucose metabolism becomes more severe as the disease progresses [87]. Neural transplantation studies have demonstrated that increased metabolic activity in the striatum correlated with an improvement of cognitive functions in HD patients [88]. Receptor binding is another measurement that can be used to assess regional resting-state activity. Numerous studies have delineated a reduction in dopamine D2 receptor binding in the striatum, which correlates with cognitive dysfunction in HD individuals [89,90]. Extrastriatal reductions in the binding of this receptor have also been observed in HD [91]. PET studies in presymptomatic mutation carriers of HD have also demonstrated a reduction in striatal dopamine receptor binding, which is a more sensitive biomarker than glucose hypometabolism or MRI volumetry [92,93]. Another approach to visualize microglial activation in PET has also been tested in the brains of HD mice and patients [94].

Reduced rCBF has been reported in the cerebellum of SCA6 patients, and the degree of reduction correlates with the disease duration [95]. Regional glucose hypometabolism in PET has been reported in patients with SCA1, SCA2, SCA3, SCA6 and SCA17 [96,97]. PET studies on presymptomatic gene carriers of SCA2 and SCA3 have also demonstrated cerebellar glucose hypometabolism without clinical signs of spinocerebellar ataxia [98,99]. Topographic brain mapping indicates a correlation between hypometabolism in the frontal cortices and the ICARS scores in a group of patients with SCA2, SCA6 or sporadic ataxia [100]. Because extrapyramidal features are often seen in some forms of polyglutamine-mediated SCAs, striatal functional imaging has also been investigated. Reduction in dopamine receptor binding in the striatum has been described in patients with SCA2, SCA3 and SCA17 [101-103]. Dopamine transporter (DAT) density, an indicator of presynaptic dopaminergic neurotransmission, is also decreased in patients with SCA2, SCA3 and SCA17 [97,104-106]. In SCA3, a reduction in DAT density is observed in the patients with no detectable parkinso-

nism and also in asymptomatic carriers [107,108]. DAT loss correlates with the ICARS score in SCA2 patients, suggesting that functional neuroimaging may provide biomarkers of polyglutamine-mediated SCAs [109].

3. Functional Imaging of Activated State

Neuroimaging studies such as functional MRI (fMRI) have been used to assess cerebral activity during cognitive tasks. fMRI measures the blood oxygen level dependent (BOLD) hemodynamic responses in the brain, which indicates neural activities. BOLD responses in the striatum have been shown to be reduced in presymptomatic HD carriers having no volumetric or behavioral abnormalities [110]. In contrast, the responses in the cortices are increased in preclinical carriers far from their estimated disease onset age, probably reflecting compensation. Neuroimaging using activation has also been applied to SCAs [111].

4. MR Spectroscopy (MRS)

MRS is a non-invasive imaging technique used to measure the relative amounts of metabolites in selected brain regions. The variables assessed in MRS include: N-acetylaspartate (NAA, marker of neuronal number); lactate (marker of hypoxia); choline (marker of membrane turnover); creatine (marker of energy metabolism) and lipid (marker of necrosis). MRS studies on presymptomatic and manifest HD individuals have shown a decrease in NAA, creatinine and choline, as well as an increase in lactate, and have demonstrated the correlation between the amounts of metabolites and disease severity [112-114].Other studies, however, have suggested the insensitivity of MRS parameters in the evaluation of the HD brain [115,116]. MRS studies on SCAs have also shown that NAA/creatine and choline/creatine levels are decreased in SCA1 and SCA2, and that the concentration of NAA in the pons correlates with the ICARS scores in SCA1 patients [80,117,118]. Although MRS may provide potential biomarkers for polyglutamine diseases, more investigations are needed for validation of the parameters.

5. Diffusion-Weighted Images

Diffusion-weighted MRI is an imaging modality to depict the local characteristics of water diffusion, which can be quantified by calculating the apparent diffusion coefficient (ADC), an indicator of water diffusion. An increased ADC in the affected regions has been reported in HD, SCA1, SCA2 and DRPLA [119-121]. A study using diffusion tensor imaging also showed that fractional anisotropy, a measure of the directional diffusion of water, was increased in the corpus callosum in early and preclinical HD individuals [122].

Clinical Measurements

Measurements from clinical assessment are also potential biomarkers, although the strategies using these parameters should overcome bias due to phenotypic variation in the patients and due to inter- and intra-rater variability. Quantitative parameters in cognitive functions have been shown to decline with disease progression, and thus have been used for the follow-up of symptomatic and presymptomatic individuals of HD [123]. Progressive impairments have been demonstrated in attention, inhibition, executive function and memory of HD individuals [124-126]. The extent of the reduction in visual processing speed and storage capacity has also been demonstrated to correlate with the disease duration in HD [127].

Eye movement is another target for identification of polyglutamine-related biomarkers. Quantitative measurements of eye movement have also shown that saccade variables, such as velocity and latency, are also potential biomarkers of disease progression in presymptomatic and early clinical stages of HD [128-130].This approach is also likely to be feasible for clinical evaluation of SCA2 patients, in whom the saccade velocity correlates with the disease duration [131]. Given that neuropathy is often seen in patients with SCAs and those with SBMA, electrophysiological parameters are alternative potential biomarkers for these diseases [132,133].

Blood and Urine Markers

Because blood is one of the most accessible human samples, serum and plasma are desirable targets for biomarker identification. Given the implication of mitochondrial damage in the pathogenesis of HD and other neurodegenerative diseases, makers of oxidative stress are plausible parameters to monitor disease progression. The serum level of 8-hydroxydeoxyguanosine (8-OHdG), an indicator of oxidative damage to DNA, has been demonstrated to be increased in HD patients and to be reduced by creatine supplementation [134]. The level of leukocyte 8-OHdG has also been reported to be elevated in HD [135]. A decrease in the levels of mitochondrial DNA from leukocytes, another marker of oxidative stress, has been shown in the blood from patients with polyglutamine diseases [136]. There are also studies demonstrating a reduced function of the A2A adenosine receptor in peripheral blood cells of individuals with HD and those with polyglutamine-mediated SCAs, although no correlation was found between the measured variable, Bmax, and the disease severity in HD [137-139]. Another study suggested an increase in the level of neural specific enolase (NSE) in the serum of symptomatic patients of SCA3 [140]. Urine is another easily accessible sample to monitor metabolic and endocrinological abnormalities in polyglutamine diseases, although human data is lacking [141].

Cerebrospinal Fluid (CSF) Markers

Cocaine- and amphetamine-regulated transcript (CART) has been shown to be elevated in CSF samples from HD patients, probably reflecting the pathogenic lesions, especially those in the hypothalamus [142]. Although the loss of orexin neurons is another indicator of hypothalamic pathology, a reduction in the CSF level of orexin has not been demonstrated in HD patients

[143,144]. F_2-isoprostane, a marker of oxidative stress, is also increased in CSF from HD patients, although the level is not correlated with disease duration [145]. CSF level of homovanillic acid has been reported to be decreased in HD, SCA1, and SCA3, reflecting an altered dopamine metabolism [146,147]. Lactate/pyruvate ratio in CSF has been shown to be increased in HD and SCA3 [148,149]. In accord with the results of gene expression analysis, inflammatory activation has also been revealed by proteomic analysis of CSF and serum from presymptomatic and manifested HD individuals [150].

Histopathological Markers

Among polyglutamine diseases, SBMA is unique in that the accumulation of causative proteins, ARs, is dependent on serum levels of testosterone. Since human AR is widely expressed in various organs, the nuclear accumulation of the pathogenic AR protein is detected not only in the central nervous system, but also in non-neuronal tissues such as the scrotal skin of SBMA patients. The degree of AR accumulation in the scrotal skin epithelial cells tends to be correlated with that in the spinal motor neurons in autopsy specimens, and it is well correlated with CAG repeat length and inversely correlated with the motor functional scale [151]. These findings indicate that the degree of AR accumulation in the scrotal skin is a biomarker with which to monitor the pathogenic processes of SBMA.

Gene Expression Profiles

Altered gene expression is a unified pathogenic feature of polyglutamine diseases, which has been reported in both animal models and patients. The polyglutamine-induced alterations in gene expression may result from various pathogenic factors such as transcriptional dysregulation and microinflammation [152,153]. Intriguingly, some of these changes are shared by the animal models of HD and DRPLA, suggesting that gene expression profiles would provide common biomarkers for various polyglutamine diseases [154]. It has been shown that gene expression changes in skeletal muscle samples from HD mice are also present in muscle biopsied from patients, indicating the possibility that non-neuronal tissues could be the target of biomarker identification [155]. This view has been exemplified by a dose-finding study of an HDAC inhibitor, sodium butyrate, in HD patients [156]. In this large-scale analysis of mRNA from blood, a significantly altered gene expression was detected in symptomatic and preclinical HD individuals, and similar changes were also detected in the postmortem HD brain. The study also demonstrated that these changes in gene expression correlated with disease progression and response to HDAC inhibition. These intriguing results suggest that expression levels of certain genes in peripheral blood could be biomarkers for polyglutamine diseases, although the sensitivity of this approach is not likely high enough for small-scale clinical trials [157].

Metabonomics/Metabolomics

Metabonomics/metabolomics is an approach to quantify the dynamic multiparametric metabolic response of living systems to pathophysiological stimuli or genetic modification, by measuring small molecular metabolites such as glucose and cholesterol. Given thermoregulatory and metabolic defects in HD mouse models, it appears legitimate to search for metabolic biomarkers for polyglutamine diseases [158,159]. Metabolite profiling by gas chromatography-time-of-flight-mass spectroscopy has demonstrated robust changes in various components of fatty acid metabolism and also those of several aliphatic amino acids in serum from HD mice and patients [160]. A decreased level of branched chain amino acids was also detected in another study on MRS analysis of the plasma from HD patients [161]. In addition, metabolite alterations have been seen in the MRS-based analysis on brain extract from SCA mice [162]. Taken together, metabolite profiling would provide important information on biomarker identification for polyglutamine diseases, although the changes in this modality may not reflect known hypotheses on the pathogenesis of neurodegeneration.

FUTURE PERSPECTIVE

Since the identification of a CAG repeat expansion within the AR gene as the cause of SBMA in 1991 [163], tremendous efforts have been made to understand and cure polyglutamine-mediated neurodegenerative disorders. It has proven to be more difficult than expected to apply disease-modifying therapies to patients, because polyglutamine diseases are relatively rare and progress slowly, and because presently available clinical parameters are not sensitive enough to detect the beneficial effects of disease-modifying therapies in interventional trials. Therefore, biomarkers reflecting the pathogenesis and severity of each disorder are needed to be identified and used as surrogate endpoints in clinical trials. In addition, biomarkers which sensitively detect presymptomatic biological changes in mutation carriers may also be applied to preventive trials, given that that early symptoms of polyglutamine diseases appear to reflect neuronal dysfunction rather than neuron loss.

Among the biomarkers so far studied, neuroimaging parameters appear to be the most reliable for symptomatic and preclinical individuals of HD. Alternative parameters, such as clinical measurements, blood markers and gene expression, are also potential biomarkers, which could be applied to one or more polyglutamine diseases, given that these markers have been identified on the basis of unified pathogenic mechanisms. It is foreseeable that a multimodal panel of biomarkers might be advantageous to define endpoints in clinical trials for polyglutamine diseases, because a combination of several markers would provide complimentary information on the different aspects of the pathogenesis. Therefore, integrated approaches are fundamental for the identification of polyglutamine-related biomarkers. An increasing number of studies have

been providing new candidate biomarkers which depict polyglutamine-related molecular changes, such as an impaired ubiquitin-proteasome system [164]. The massive expansion of molecular imaging studies is also expected to aid in identifying novel molecular markers of polyglutamine diseases.

Reliance on biomarkers, however, can be misleading, because surrogate endpoints may not accurately predict the actual effects of therapeutic interventions. The limitation of biomarkers has been illuminated in various clinical trials [165]. In certain circumstances, a therapeutic intervention may improve clinical outcome measurements without any changes of biomarkers. Conversely, some therapeutics might affect surrogate endpoints with no amelioration of true clinical endpoints. These false-positive and false-negative possibilities underscore the cautious exploitation of biomarkers in therapy development. It is thus of critical importance to validate candidate biomarkers for polyglutamine diseases in both cross-sectional and longitudinal studies. Integrated approaches, such as long-term surveillance and phase 4 clinical trials, would reinforce the plausibility of biomarkers.

ACKNOWLEDGEMENTS

This work was supported by the Program for Improvement of Research Environment for Young Researchers from Special Coordination Funds for Promoting Science and Technology (SCF) commissioned by the Ministry of Education, Culture, Sports, Science and Technology of Japan, a Center-of-Excellence (COE) grant from the Ministry of Education, Culture, Sports, Science and Technology, Japan, and grants from the Ministry of Health, Labor and Welfare, Japan.

REFERENCES

[1] Walker, F.O. (2007) *Lancet*, **369**, 218-228.
[2] Fischbeck, K.H. (1997) *J. Inherit. Metab. Dis.*, **20**, 152-158.
[3] Tsuji, S. (2002) *Adv. Neurol.*, **89**, 231-239.
[4] Naito, H. and Oyanagi, S. (1982) *Neurology*, **32**, 798-807.
[5] Burke, J.R., Wingfield, M.S., Lewis, K.E., Roses, A.D., Lee, J.E., Hulette, C., Pericak-Vance, M.A. and Vance, J.M. (1994) *Nat. Genet.*, **7**, 521-524.
[6] Kennedy, W.R., Alter, M. and Sung, J.H. (1968) *Neurology*, **18**, 671-680.
[7] Atsuta, N., Watanabe, H., Ito, M., Banno, H., Suzuki, K., Katsuno, M., Tanaka, F., Tamakoshi, A. and Sobue, G. (2006) *Brain*, **129**, 1446-1455.
[8] Ikeuchi, T., Koide, R., Onodera, O., Tanaka, H., Oyake, M., Takano, H. and Tsuji, S. (1995) *Clin. Neurosci.*, **3**, 23-27.
[9] Zoghbi, H.Y. and Orr, H.T. (2000) *Annu. Rev. Neurosci.*, **23**, 217-247.
[10] Nakamura, K., Jeong, S.Y., Uchihara, T., Anno, M., Nagashima, K., Nagashima, T., Ikeda, S., Tsuji, S. and Kanazawa, I. (2001) *Hum. Mol. Genet.*, **10**, 1441-1448.
[11] Gatchel, J.R. and Zoghbi, H.Y. (2005) *Nat. Rev. Genet.*, **6**, 743-755.
[12] Schmidt, B.J., Greenberg, C.R., Allingham-Hawkins, D.J. and Spriggs, E.L. (2002) *Neurology*, **59**, 770-772.
[13] Paulsen, J.S., Hayden, M., Stout, J.C., Langbehn, D.R., Aylward, E., Ross, C.A., Guttman, M., Nance, M., Kieburtz, K., Oakes, D., Shoulson, I., Kayson, E., Johnson, S. and Penziner, E. (2006) *Arch. Neurol.*, **63**, 883-890.
[14] Podolsky, S., Leopold, N. A. and Sax, D.S. (1972) *Lancet*, **1**, 1356-1358.
[15] Sinnreich, M. and Klein, C.J. (2004) *Arch. Neurol.*, **61**, 1324-1326.
[16] Bates, G. (2003) *Lancet*, **361**, 1642-1644.
[17] Martindale, D., Hackam, A., Wieczorek, A., Ellerby, L., Wellington, C., Mccutcheon, K., Singaraja, R., Kazemi-Esfarjani, P., Devon, R., Kim, S.U., Bredesen, D. E., Tufaro, F. and Hayden, M. R. (1998) *Nat. Genet.*, **18**, 150-154.
[18] Arrasate, M., Mitra, S., Schweitzer, E.S., Segal, M.R. and Finkbeiner, S. (2004) *Nature*, **431**, 805-810.
[19] Yamada, M., Wood, J.D., Shimohata, T., Hayashi, S., Tsuji, S., Ross, C.A. and Takahashi, H. (2001) *Ann. Neurol.*, **49**, 14-23.
[20] Adachi, H., Katsuno, M., Minamiyama, M., Waza, M., Sang, C., Nakagomi, Y., Kobayashi, Y., Tanaka, F., Doyu, M., Inukai, A., Yoshida, M., Hashizume, Y. and Sobue, G. (2005) *Brain*, **128**, 659-670.
[21] Taylor, J.P., Hardy, J. and Fischbeck, K.H. (2002) *Science*, **296**, 1991-1995.
[22] Michalik, A. and Van Broeckhoven, C. (2004) *Neurobiol. Dis.*, **16**, 202-211.
[23] Muchowski, P.J. and Wacker, J.L. (2005) *Nat. Rev. Neurosci.*, **6**, 11-22.
[24] Nagai, Y., Inui, T., Popiel, H.A., Fujikake, N., Hasegawa, K., Urade, Y., Goto, Y., Naiki, H. and Toda, T. (2007) *Nat. Struct. Mol. Biol.*, **14**, 332-340.
[25] Sugars, K.L. and Rubinsztein, D.C. (2003) *Trends Genet.*, **19**, 233-238.
[26] Nucifora, F.C., Jr., Sasaki, M., Peters, M.F., Huang, H., Cooper, J.K., Yamada, M., Takahashi, H., Tsuji, S., Troncoso, J., Dawson, V.L., Dawson, T.M. and Ross, C.A. (2001) *Science*, **291**, 2423-2428.
[27] Steffan, J.S., Kazantsev, A., Spasic-Boskovic, O., Greenwald, M., Zhu, Y. Z., Gohler, H., Wanker, E.E., Bates, G.P., Housman, D.E. and Thompson, L.M. (2000) *Proc. Natl. Acad. Sci. USA*, **97**, 6763-6768.
[28] Panov, A.V., Gutekunst, C.A., Leavitt, B.R., Hayden, M.R., Burke, J.R., Strittmatter, W.J. and Greenamyre, J.T. (2002) *Nat. Neurosci.*, **5**, 731-736.
[29] Browne, S.E., Bowling, A.C., Macgarvey, U., Baik, M.J., Berger, S.C., Muqit, M.M., Bird, E.D. and Beal, M.F. (1997) *Ann. Neurol.*, **41**, 646-653.
[30] Horton, T.M., Graham, B.H., Corral-Debrinski, M., Shoffner, J.M., Kaufman, A.E., Beal, M.F. and Wallace, D.C. (1995) *Neurology*, **45**, 1879-1883.
[31] Cui, L., Jeong, H., Borovecki, F., Parkhurst, C.N., Tanese, N. and Krainc, D. (2006) *Cell*, **127**, 59-69.
[32] Sanchez, I., Xu, C. J., Juo, P., Kakizaka, A., Blenis, J. and Yuan, J. (1999) *Neuron*, **22**, 623-633.
[33] Ona, V.O., Li, M., Vonsattel, J.P., Andrews, L. J., Khan, S.Q., Chung, W. M., Frey, A.S., Menon, A.S., Li, X.J., Stieg, P.E., Yuan, J., Penney, J.B., Young, A.B., Cha, J.H. and Friedlander, R.M. (1999) *Nature*, **399**, 263-267.
[34] Gafni, J. and Ellerby, L.M. (2002) *J. Neurosci.*, **22**, 4842-4849.
[35] Gunawardena, S., Her, L.S., Brusch, R.G., Laymon, R.A., Niesman, I.R., Gordesky-Gold, B., Sintasath, L., Bonini, N.M. and Goldstein, L.S. (2003) *Neuron*, **40**, 25-40.
[36] Szebenyi, G., Morfini, G.A., Babcock, A., Gould, M., Selkoe, K., Stenoien, D.L., Young, M., Faber, P.W., Macdonald, M.E., Mcphaul, M.J. and Brady, S.T. (2003) *Neuron*, **40**, 41-52.
[37] Katsuno, M., Adachi, H., Minamiyama, M., Waza, M., Tokui, K., Banno, H., Suzuki, K., Onoda, Y., Tanaka, F., Doyu, M. and Sobue, G. (2006) *J. Neurosci.*, **26**, 12106-12117.
[38] Morfini, G., Pigino, G., Szebenyi, G., You, Y., Pollema, S. and Brady, S.T. (2006) *Nat. Neurosci.*, **9**, 907-916.
[39] Mangiarini, L., Sathasivam, K., Seller, M., Cozens, B., Harper, A., Hetherington, C., Lawton, M., Trottier, Y., Lehrach, H., Davies, S.W. and Bates, G. P. (1996) *Cell*, **87**, 493-506.
[40] Yamamoto, A., Lucas, J.J. and Hen, R. (2000) *Cell*, **101**, 57-66.

[41] Zu, T., Duvick, L.A., Kaytor, M.D., Berlinger, M.S., Zoghbi, H.Y., Clark, H.B. and Orr, H.T. (2004) *J. Neurosci.*, **24**, 8853-8861.
[42] Vonsattel, J.P., Myers, R.H., Stevens, T.J., Ferrante, R.J., Bird, E.D. and Richardson, E.P., Jr. (1985) *J. Neuropathol. Exp. Neurol.*, **44**, 559-577.
[43] Heiser, V., Engemann, S., Brocker, W., Dunkel, I., Boeddrich, A., Waelter, S., Nordhoff, E., Lurz, R., Schugardt, N., Rautenberg, S., Herhaus, C., Barnickel, G., Bottcher, H., Lehrach, H. and Wanker, E.E. (2002) *Proc. Natl. Acad. Sci. USA*, **99**(Suppl 4), 16400-16406.
[44] Tanaka, M., Machida, Y., Niu, S., Ikeda, T., Jana, N. R., Doi, H., Kurosawa, M., Nekooki, M. and Nukina, N. (2004) *Nat. Med.*, **10**, 148-154.
[45] Katsuno, M., Adachi, H., Kume, A., Li, M., Nakagomi, Y., Niwa, H., Sang, C., Kobayashi, Y., Doyu, M. and Sobue, G. (2002) *Neuron*, **35**, 843-854.
[46] Takeyama, K., Ito, S., Yamamoto, A., Tanimoto, H., Furutani, T., Kanuka, H., Miura, M., Tabata, T. and Kato, S. (2002) *Neuron*, **35**, 855-864.
[47] Chevalier-Larsen, E. S., O'brien, C.J., Wang, H., Jenkins, S.C., Holder, L., Lieberman, A.P. and Merry, D. E. (2004) *J. Neurosci.*, **24**, 4778-4786.
[48] Katsuno, M., Adachi, H., Doyu, M., Minamiyama, M., Sang, C., Kobayashi, Y., Inukai, A. and Sobue, G. (2003) *Nat. Med.*, **9**, 768-773.
[49] Cummings, C.J., Sun, Y., Opal, P., Antalffy, B., Mestril, R., Orr, H.T., Dillmann, W.H. and Zoghbi, H.Y. (2001) *Hum. Mol. Genet.*, **10**, 1511-1518.
[50] Bailey, C.K., Andriola, I.F., Kampinga, H.H. and Merry, D.E. (2002) *Hum. Mol. Genet.*, **11**, 515-523.
[51] Adachi, H., Katsuno, M., Minamiyama, M., Sang, C., Pagoulatos, G., Angelidis, C., Kusakabe, M., Yoshiki, A., Kobayashi, Y., Doyu, M. and Sobue, G. (2003) *J. Neurosci.*, **23**, 2203-2211.
[52] Adachi, H., Waza, M., Tokui, K., Katsuno, M., Minamiyama, M., Tanaka, F., Doyu, M. and Sobue, G. (2007) *J. Neurosci.*, **27**, 5115-5126.
[53] Katsuno, M., Sang, C., Adachi, H., Minamiyama, M., Waza, M., Tanaka, F., Doyu, M. and Sobue, G. (2005) *Proc. Natl. Acad. Sci. USA*, **102**, 16801-16806.
[54] Herbst, M. and Wanker, E. E. (2007) *Neurodegener. Dis.*, **4**, 254-260.
[55] Waza, M., Adachi, H., Katsuno, M., Minamiyama, M., Sang, C., Tanaka, F., Inukai, A., Doyu, M. and Sobue, G. (2005) *Nat. Med.*, **11**, 1088-1095.
[56] Thomas, M., Harrell, J.M., Morishima, Y., Peng, H.M., Pratt, W.B. and Lieberman, A.P. (2006) *Hum. Mol. Genet.*, **15**, 1876-1883.
[57] Ravikumar, B., Vacher, C., Berger, Z., Davies, J.E., Luo, S., Oroz, L.G., Scaravilli, F., Easton, D.F., Duden, R., O'kane, C.J. and Rubinsztein, D.C. (2004) *Nat. Genet.*, **36**, 585-595.
[58] Minamiyama, M., Katsuno, M., Adachi, H., Waza, M., Sang, C., Kobayashi, Y., Tanaka, F., Doyu, M., Inukai, A. and Sobue, G. (2004) *Hum. Mol. Genet.*, **13**, 1183-1192.
[59] Ying, M., Xu, R., Wu, X., Zhu, H., Zhuang, Y., Han, M. and Xu, T. (2006) *J. Biol. Chem.*, **281**, 12580-12586.
[60] Ferrante, R.J., Kubilus, J.K., Lee, J., Ryu, H., Beesen, A., Zucker, B., Smith, K., Kowall, N. W., Ratan, R. R., Luthi-Carter, R. and Hersch, S. M. (2003) *J. Neurosci.*, **23**, 9418-9427.
[61] Hockly, E., Richon, V.M., Woodman, B., Smith, D.L., Zhou, X., Rosa, E., Sathasivam, K., Ghazi-Noori, S., Mahal, A., Lowden, P.A., Steffan, J.S., Marsh, J.L., Thompson, L.M., Lewis, C.M., Marks, P.A. and Bates, G.P. (2003) *Proc. Natl. Acad. Sci. USA*, **100**, 2041-2046.
[62] Gardian, G., Browne, S.E., Choi, D.K., Klivenyi, P., Gregorio, J., Kubilus, J. K., Ryu, H., Langley, B., Ratan, R.R., Ferrante, R.J. and Beal, M.F. (2005) *J. Biol. Chem.*, **280**, 556-563.
[63] Di Prospero, N.A. and Fischbeck, K.H. (2005) *Nat. Rev. Genet.*, **6**, 756-765.
[64] Biomarkers definitions working group (2001) *Clin. Pharmacol. Ther.*, **69**, 89-95.
[65] Marder, K., Zhao, H., Myers, R.H., Cudkowicz, M., Kayson, E., Kieburtz, K., Orme, C., Paulsen, J., Penney, J.B., Jr., Siemers, E. and Shoulson, I. (2000) *Neurology*, **54**, 452-458.
[66] Henley, S.M., Bates, G.P. and Tabrizi, S.J. (2005) *Curr. Opin. Neurol.*, **18**, 698-705.
[67] Rosas, H.D., Feigin, A.S. and Hersch, S.M. (2004) *NeuroRx.*, **1**, 263-272.
[68] Montoya, A., Price, B.H., Menear, M. and Lepage, M. (2006) *J. Psychiatry. Neurosci.*, **31**, 21-29.
[69] Harris, G.J., Pearlson, G.D., Peyser, C.E., Aylward, E.H., Roberts, J., Barta, P.E., Chase, G.A. and Folstein, S.E. (1992) *Ann. Neurol.*, **31**, 69-75.
[70] Brandt, J., Bylsma, F.W., Aylward, E.H., Rothlind, J. and Gow, C.A. (1995) *J. Clin. Exp. Neuropsychol.*, **17**, 868-877.
[71] Aylward, E.H., Sparks, B.F., Field, K.M., Yallapragada, V., Shpritz, B.D., Rosenblatt, A., Brandt, J., Gourley, L.M., Liang, K., Zhou, H., Margolis, R. L. and Ross, C. A. (2004) *Neurology*, **63**, 66-72.
[72] Rosas, H.D., Koroshetz, W.J., Chen, Y.I., Skeuse, C., Vangel, M., Cudkowicz, M.E., Caplan, K., Marek, K., Seidman, L. J., Makris, N., Jenkins, B. G. and Goldstein, J. M. (2003) *Neurology*, **60**, 1615-1620.
[73] Henley, S.M., Frost, C., Macmanus, D.G., Warner, T.T., Fox, N.C. and Tabrizi, S.J. (2006) *Neurology*, **67**, 694-696.
[74] Kassubek, J., Juengling, F.D., Kioschies, T., Henkel, K., Karitzky, J., Kramer, B., Ecker, D., Andrich, J., Saft, C., Kraus, P., Aschoff, A.J., Ludolph, A.C. and Landwehrmeyer, G.B. (2004) *J. Neurol. Neurosurg. Psychiatry*, **75**, 213-220.
[75] Kipps, C. M., Duggins, A. J., Mahant, N., Gomes, L., Ashburner, J. and Mccusker, E. A. (2005) *J. Neurol. Neurosurg. Psychiatry*, **76**, 650-655.
[76] Klockgether, T., Skalej, M., Wedekind, D., Luft, A. R., Welte, D., Schulz, J. B., Abele, M., Burk, K., Laccone, F., Brice, A. and Dichgans, J. (1998) *Brain*, **121**(Pt 9), 1687-1693.
[77] Murata, Y., Kawakami, H., Yamaguchi, S., Nishimura, M., Kohriyama, T., Ishizaki, F., Matsuyama, Z., Mimori, Y. and Nakamura, S. (1998) *Arch. Neurol.*, **55**, 1348-1352.
[78] Etchebehere, E.C., Cendes, F., Lopes-Cendes, I., Pereira, J.A., Lima, M. C., Sansana, C.R., Silva, C.A., Camargo, M.F., Santos, A.O., Ramos, C.D. and Camargo, E.E. (2001) *Arch. Neurol.*, **58**, 1257-1263.
[79] Bang, O.Y., Lee, P.H., Kim, S.Y., Kim, H.J. and Huh, K. (2004) *J. Neurol. Neurosurg. Psychiatry*, **75**, 1452-1456.
[80] Guerrini, L., Lolli, F., Ginestroni, A., Belli, G., Della Nave, R., Tessa, C., Foresti, S., Cosottini, M., Piacentini, S., Salvi, F., Plasmati, R., De Grandis, D., Siciliano, G., Filla, A. and Mascalchi, M. (2004) *Brain*, **127**, 1785-1795.
[81] Richter, S., Dimitrova, A., Maschke, M., Gizewski, E., Beck, A., Aurich, V. and Timmann, D. (2005) *Eur. Neurol.*, **54**, 23-27.
[82] Lukas, C., Schols, L., Bellenberg, B., Rub, U., Przuntek, H., Schmid, G., Koster, O. and Suchan, B. (2006) *Neurosci. Lett.*, **408**, 230-235.
[83] Tanahashi, N., Meyer, J.S., Ishikawa, Y., Kandula, P., Mortel, K.F., Rogers, R.L., Gandhi, S. and Walker, M. (1985) *Arch. Neurol.*, **42**, 1169-1175.
[84] Hasselbalch, S.G., Oberg, G., Sorensen, S.A., Andersen, A.R., Waldemar, G., Schmidt, J.F., Fenger, K. and Paulson, O.B. (1992) *J. Neurol. Neurosurg. Psychiatry*, **55**, 1018-1023.
[85] Berent, S., Giordani, B., Lehtinen, S., Markel, D., Penney, J.B., Buchtel, H. A., Starosta-Rubinstein, S., Hichwa, R. and Young, A.B. (1988) *Ann. Neurol.*, **23**, 541-546.
[86] Kuwert, T., Lange, H.W., Langen, K.J., Herzog, H., Aulich, A. and Feinendegen, L.E. (1990) *Brain*, **113**(Pt 5), 1405-1423.
[87] Kremer, B., Clark, C.M., Almqvist, E.W., Raymond, L.A., Graf, P., Jacova, C., Mezei, M., Hardy, M.A., Snow, B., Martin, W. and Hayden, M.R. (1999) *Neurology*, **53**, 1000-1011.
[88] Bachoud-Levi, A., Bourdet, C., Brugieres, P., Nguyen, J.P., Grandmougin, T., Haddad, B., Jeny, R., Bartolomeo, P., Boisse, M.F., Barba, G.D., Degos, J.D., Ergis, A.M., Lefaucheur, J.P., Lisovoski, F., Pailhous, E., Remy, P., Palfi, S., Defer, G.L., Cesaro, P., Hantraye, P. and Peschanski, M. (2000) *Exp. Neurol.*, **161**, 194-202.

[89] Backman, L., Robins-Wahlin, T.B., Lundin, A., Ginovart, N. and Farde, L. (1997) *Brain*, **120**(Pt 12), 2207-2217.
[90] Kunig, G., Leenders, K.L., Sanchez-Pernaute, R., Antonini, A., Vontobel, P., Verhagen, A. and Gunther, I. (2000) *Ann. Neurol.*, **47**, 644-648.
[91] Pavese, N., Andrews, T.C., Brooks, D.J., Ho, A.K., Rosser, A.E., Barker, R. A., Robbins, T.W., Sahakian, B.J., Dunnett, S.B. and Piccini, P. (2003) *Brain*, **126**, 1127-1135.
[92] Lawrence, A.D., Weeks, R.A., Brooks, D.J., Andrews, T.C., Watkins, L.H., Harding, A.E., Robbins, T.W. and Sahakian, B.J. (1998) *Brain*, **121**(Pt 7), 1343-1355.
[93] Van Oostrom, J.C., Maguire, R.P., Verschuuren-Bemelmans, C.C., Veenma-Van Der Duin, L., Pruim, J., Roos, R. A. and Leenders, K.L.(2005) *Neurology*, **65**, 941-943.
[94] Pavese, N., Gerhard, A., Tai, Y.F., Ho, A.K., Turkheimer, F., Barker, R.A., Brooks, D.J. and Piccini, P. (2006) *Neurology*, **66**, 1638-1643.
[95] Honjo, K., Ohshita, T., Kawakami, H., Naka, H., Imon, Y., Maruyama, H., Mimori, Y. and Matsumoto, M. (2004) *Arch. Neurol.*, **61**, 933-937.
[96] Minnerop, M., Joe, A., Lutz, M., Bauer, P., Urbach, H., Helmstaedter, C., Reinhardt, M., Klockgether, T. and Wullner, U. (2005) *Ann. Neurol.*, **58**, 490-491.
[97] Wullner, U., Reimold, M., Abele, M., Burk, K., Minnerop, M., Dohmen, B.M., Machulla, H.J., Bares, R. and Klockgether, T. (2005) *Arch. Neurol.*, **62**, 1280-1285.
[98] Soong, B.W. and Liu, R.S. (1998) *J. Neurol. Neurosurg. Psychiatry*, **64**, 499-504.
[99] Inagaki, A., Iida, A., Matsubara, M. and Inagaki, H. (2005) *Eur. J. Neurol.*, **12**, 725-728.
[100] Wang, P.S., Liu, R.S., Yang, B.H. and Soong, B.W. (2007) *J. Neurol.*, **254**, 722-728.
[101] Taniwaki, T., Sakai, T., Kobayashi, T., Kuwabara, Y., Otsuka, M., Ichiya, Y., Masuda, K. and Goto, I. (1997) *J. Neurol. Sci.*, **145**, 63-67.
[102] Furtado, S., Farrer, M., Tsuboi, Y., Klimek, M.L., De La Fuente-Fernandez, R., Hussey, J., Lockhart, P., Calne, D.B., Suchowersky, O., Stoessl, A.J. and Wszolek, Z.K. (2002) *Neurology*, **59**, 1625-1627.
[103] Lin, I.S., Wu, R.M., Lee-Chen, G.J., Shan, D.E. and Gwinn-Hardy, K. (2007) *Parkinsonism Relat. Disord.*, **13**, 246-249.
[104] Lu, C.S., Wu Chou, Y.H., Yen, T.C., Tsai, C.H., Chen, R.S. and Chang, H.C. (2002) *Mov. Disord.*, **17**, 1046-1051.
[105] Boesch, S. M., Donnemiller, E., Muller, J., Seppi, K., Weirich-Schwaiger, H., Poewe, W. and Wenning, G. K. (2004) *Mov. Disord.*, **19**, 1320-1325.
[106] Salvatore, E., Varrone, A., Sansone, V., Nolano, M., Bruni, A.C., De Rosa, A., Santoro, L., Pappata, S., Filla, A. and De Michele, G. (2006) *Mov. Disord.*, **21**, 872-875.
[107] Yen, T.C., Lu, C.S., Tzen, K.Y., Wey, S.P., Chou, Y.H., Weng, Y.H., Kao, P.F. and Ting, G. (2000) *J. Nucl. Med.*, **41**, 994-998.
[108] Yen, T.C., Tzen, K.Y., Chen, M.C., Chou, Y.H., Chen, R.S., Chen, C.J., Wey, S.P., Ting, G. and Lu, C.S. (2002) *J. Nucl. Med.*, **43**, 153-159.
[109] Varrone, A., Salvatore, E., De Michele, G., Barone, P., Sansone, V., Pellecchia, M.T., Castaldo, I., Coppola, G., Brunetti, A., Salvatore, M., Pappata, S. and Filla, A. (2004) *Ann. Neurol.*, **55**, 426-430.
[110] Paulsen, J.S., Zimbelman, J.L., Hinton, S.C., Langbehn, D.R., Leveroni, C. L., Benjamin, M. L., Reynolds, N. C. and Rao, S. M. (2004) *AJNR Am. J. Neuroradiol.*, **25**, 1715-1721.
[111] Sidtis, J.J., Gomez, C., Groshong, A., Strother, S.C. and Rottenberg, D. A. (2006) *Neuroimage.*, **31**, 246-254.
[112] Jenkins, B.G., Rosas, H.D., Chen, Y.C., Makabe, T., Myers, R., Macdonald, M., Rosen, B.R., Beal, M. F. and Koroshetz, W.J. (1998) *Neurology*, **50**, 1357-1365.
[113] Sanchez-Pernaute, R., Garcia-Segura, J.M., Del Barrio Alba, A., Viano, J. and De Yebenes, J.G. (1999) *Neurology*, **53**, 806-812.
[114] Gomez-Anson, B., Alegret, M., Munoz, E., Sainz, A., Monte, G.C. and Tolosa, E. (2007) *Neurology*, **68**, 906-910.
[115] Reynolds, N.C., Jr., Prost, R. W. and Mark, L.P. (2005) *Brain Res.*, **1031**, 82-89.
[116] Van Oostrom, J.C., Sijens, P.E., Roos, R.A. and Leenders, K.L. (2007) *Brain Res.*, **1168**, 67-71.
[117] Mascalchi, M., Tosetti, M., Plasmati, R., Bianchi, M.C., Tessa, C., Salvi, F., Frontali, M., Valzania, F., Bartolozzi, C. and Tassinari, C.A. (1998) *Ann. Neurol.*, **43**, 244-252.
[118] Boesch, S. M., Wolf, C., Seppi, K., Felber, S., Wenning, G. K. and Schocke, M. (2007) *J. Magn. Reson. Imaging*, **25**, 564-569.
[119] Mascalchi, M., Lolli, F., Della Nave, R., Tessa, C., Petralli, R., Gavazzi, C., Politi, L.S., Macucci, M., Filippi, M. and Piacentini, S. (2004) *Radiology*, **232**, 867-873.
[120] Della Nave, R., Foresti, S., Tessa, C., Moretti, M., Ginestroni, A., Gavazzi, C., Guerrini, L., Salvi, F., Piacentini, S. and Mascalchi, M. (2004) *Neuroimage.*, **22**, 698-705.
[121] Kin, T., Hirano, M., Taoka, T., Furiya, Y., Kataoka, H., Kichikawa, K. and Ueno, S. (2006) *AJNR Am. J. Neuroradiol.*, **27**, 1463-1466.
[122] Rosas, H.D., Tuch, D.S., Hevelone, N.D., Zaleta, A. K., Vangel, M., Hersch, S.M. and Salat, D.H. (2006) *Mov. Disord.*, **21**, 1317-1325.
[123] Lemiere, J., Decruyenaere, M., Evers-Kiebooms, G., Vandenbussche, E. and Dom, R. (2004) *J. Neurol.*, **251**, 935-942.
[124] Lawrence, A.D., Hodges, J.R., Rosser, A.E., Kershaw, A., Ffrench-Constant, C., Rubinsztein, D. C., Robbins, T.W. and Sahakian, B. J. (1998) *Brain*, **121**(Pt 7), 1329-1341.
[125] Ho, A.K., Sahakian, B.J., Brown, R.G., Barker, R.A., Hodges, J.R., Ane, M.N., Snowden, J., Thompson, J., Esmonde, T., Gentry, R., Moore, J. W. and Bodner, T. (2003) *Neurology*, **61**, 1702-1706.
[126] Farrow, M., Churchyard, A., Chua, P., Bradshaw, J.L., Chiu, E. and Georgiou-Karistianis, N. (2007) *J. Clin. Exp. Neuropsychol.*, **29**, 235-246.
[127] Finke, K., Bublak, P., Dose, M., Muller, H.J. and Schneider, W.X. (2006) *Brain*, **129**, 1137-1151.
[128] Blekher, T., Johnson, S.A., Marshall, J., White, K., Hui, S., Weaver, M., Gray, J., Yee, R., Stout, J.C., Beristain, X., Wojcieszek, J. and Foroud, T. (2006) *Neurology*, **67**, 394-399.
[129] Golding, C.V., Danchaivijitr, C., Hodgson, T.L., Tabrizi, S.J. and Kennard, C. (2006) *Neurology*, **67**, 485-487.
[130] Antoniades, C.A., Altham, P.M., Mason, S.L., Barker, R. A. and Carpenter, R. (2007) *Neuroreport.*, **18**, 1133-1136.
[131] Seifried, C., Velazquez-Perez, L., Santos-Falcon, N., Abele, M., Ziemann, U., Almaguer, L. E., Martinez-Gongora, E., Sanchez-Cruz, G., Canales, N., Perez-Gonzalez, R., Velazquez-Manresa, M., Viebahn, B., Stuckrad-Barre, S., Klockgether, T., Fetter, M. and Auburger, G. (2005) *Ann. N. Y. Acad. Sci.*, **1039**, 524-527.
[132] Schols, L., Linnemann, C. and Globas, C. (2007) *Cerebellum*, 1-6.
[133] Suzuki, K., Katsuno, M., Banno, H., Takeuchi, Y., Atsuta, N., Ito, M., Watanabe, H., Yamashita, F., Hori, N., Nakamura, T., Tanaka, F., Hirayama, M., and Sobue, G. *Brain*, **131**, 229-239.
[134] Hersch, S.M., Gevorkian, S., Marder, K., Moskowitz, C., Feigin, A., Cox, M., Como, P., Zimmerman, C., Lin, M., Zhang, L., Ulug, A.M., Beal, M. F., Matson, W., Bogdanov, M., Ebbel, E., Zaleta, A., Kaneko, Y., Jenkins, B., Hevelone, N., Zhang, H., Yu, H., Schoenfeld, D., Ferrante, R. and Rosas, H.D. (2006) *Neurology*, **66**, 250-252.
[135] Chen, C.M., Wu, Y.R., Cheng, M.L., Liu, J.L., Lee, Y.M., Lee, P.W., Soong, B.W. and Chiu, D.T. (2007) *Biochem. Biophys. Res. Commun.*, **359**, 335-340.
[136] Liu, C.S., Cheng, W.L., Kuo, S.J., Li, J.Y., Soong, B. W. and Wei, Y.H. (2007) *J. Neurol. Sci.* [in press].
[137] Varani, K., Abbracchio, M.P., Cannella, M., Cislaghi, G., Giallonardo, P., Mariotti, C., Cattabriga, E., Cattabeni, F., Borea, P. A., Squitieri, F. and Cattaneo, E. (2003) *FASEB J.*, **17**, 2148-2150.
[138] Maglione, V., Giallonardo, P., Cannella, M., Martino, T., Frati, L. and Squitieri, F. (2005) *Am. J. Med. Genet. B. Neuropsychiatr. Genet.*, **139**, 101-105.
[139] Varani, K., Bachoud-Levi, A.C., Mariotti, C., Tarditi, A., Abbracchio, M. P., Gasperi, V., Borea, P. A., Dolbeau, G., Ge-

llera, C., Solari, A., Rosser, A., Naji, J., Handley, O., Maccarrone, M., Peschanski, M., Didonato, S. and Cattaneo, E. (2007) *Neurobiol. Dis.*, **27**, 36-43.
[140] Tort, A.B., Portela, L.V., Rockenbach, I.C., Monte, T.L., Pereira, M.L., Souza, D.O., Rieder, C.R. and Jardim, L.B. (2005) *Clin. Chim. Acta.*, **351**, 143-148.
[141] Petersen, A. and Bjorkqvist, M. (2006) *Eur. J. Neurosci.*, **24**, 961-967.
[142] Bjorkqvist, M., Leavitt, B.R., Nielsen, J. E., Landwehrmeyer, B., Ecker, D., Mulder, H., Brundin, P. and Petersen, A. (2007) *Mov. Disord.* [in press].
[143] Petersen, A., Gil, J., Maat-Schieman, M. L., Bjorkqvist, M., Tanila, H., Araujo, I. M., Smith, R., Popovic, N., Wierup, N., Norlen, P., Li, J. Y., Roos, R. A., Sundler, F., Mulder, H. and Brundin, P. (2005) *Hum. Mol. Genet.*, **14**, 39-47.
[144] Bjorkqvist, M., Petersen, A., Nielsen, J., Ecker, D., Mulder, H., Hayden, M.R., Landwehrmeyer, B., Brundin, P. and Leavitt, B.R. (2006) *Clin. Genet.*, **70**, 78-79.
[145] Montine, T.J., Beal, M.F., Robertson, D., Cudkowicz, M.E., Biaggioni, I., O'Donnell, H., Zackert, W.E., Roberts, L.J. and Morrow, J.D. (1999) *Neurology*, **52**, 1104-1105.
[146] Garcia Ruiz, P.J., Mena, M.A., Sanchez Bernardos, V., Diaz Neira, W., Gimenez Roldan, S., Benitez, J. and Garcia de Yebenes, J. (1995) *Clin. Neuropharmacol.*, **18**, 58-63.
[147] Higgins, J.J., Harvey-White, J.D., Nee, L.E., Colli, M.J., Grossi, T.A. and Kopin, I.J. (1996) *J. Neurol. Neurosurg. Psychiatry*, **61**, 591-595.
[148] Matsuishi, T., Sakai, T., Naito, E., Nagamitsu, S., Kuroda, Y., Iwashita, H. and Kato, H. (1996) *Acta Neurol. Scand.*, **93**, 72-75.
[149] Koroshetz, W.J., Jenkins, B.G., Rosen, B.R. and Beal, M.F. (1997) *Ann. Neurol.*, **41**, 160-165.
[150] Dalrymple, A., Wild, E. J., Joubert, R., Sathasivam, K., Bjorkqvist, M., Petersen, A., Jackson, G.S., Isaacs, J.D., Kristiansen, M., Bates, G.P., Leavitt, B.R., Keir, G., Ward, M. and Tabrizi, S.J. (2007) *J. Proteome Res.*, **6**, 2833-2840.
[151] Banno, H., Adachi, H., Katsuno, M., Suzuki, K., Atsuta, N., Watanabe, H., Tanaka, F., Doyu, M. and Sobue, G. (2006) *Ann. Neurol.*, **59**, 520-526.
[152] Dunah, A.W., Jeong, H., Griffin, A., Kim, Y.M., Standaert, D.G., Hersch, S.M., Mouradian, M.M., Young, A.B., Tanese, N. and Krainc, D. (2002) *Science*, **296**, 2238-2243.
[153] Evert, B.O., Vogt, I.R., Kindermann, C., Ozimek, L., De Vos, R.A., Brunt, E.R., Schmitt, I., Klockgether, T. and Wullner, U. (2001) *J. Neurosci.*, **21**, 5389-5396.
[154] Luthi-Carter, R., Strand, A.D., Hanson, S. A., Kooperberg, C., Schilling, G., La Spada, A.R., Merry, D.E., Young, A.B., Ross, C.A., Borchelt, D. R. and Olson, J.M. (2002) *Hum. Mol. Genet.*, **11**, 1927-1937.
[155] Strand, A.D., Aragaki, A.K., Shaw, D., Bird, T., Holton, J., Turner, C., Tapscott, S.J., Tabrizi, S.J., Schapira, A.H., Kooperberg, C. and Olson, J. M. (2005) *Hum. Mol. Genet.*, **14**, 1863-1876.
[156] Borovecki, F., Lovrecic, L., Zhou, J., Jeong, H., Then, F., Rosas, H.D., Hersch, S M., Hogarth, P., Bouzou, B., Jensen, R.V. and Krainc, D. (2005) *Proc. Natl. Acad. Sci. USA*, **102**, 11023-11028.
[157] Runne, H., Kuhn, A., Wild, E.J., Pratyaksha, W., Kristiansen, M., Isaacs, J.D., Regulier, E., Delorenzi, M., Tabrizi, S. J. and Luthi-Carter, R. (2007) *Proc. Natl. Acad. Sci. USA*, **104**, 14424-14429.
[158] Valenza, M., Rigamonti, D., Goffredo, D., Zuccato, C., Fenu, S., Jamot, L., Strand, A., Tarditi, A., Woodman, B., Racchi, M., Mariotti, C., Di Donato, S., Corsini, A., Bates, G., Pruss, R., Olson, J.M., Sipione, S., Tartari, M. and Cattaneo, E. (2005) *J. Neurosci.*, **25**, 9932-9939.
[159] Weydt, P., Pineda, V.V., Torrence, A.E., Libby, R.T., Satterfield, T.F., Lazarowski, E.R., Gilbert, M.L., Morton, G.J., Bammler, T.K., Strand, A.D., Cui, L., Beyer, R.P., Easley, C.N., Smith, A.C., Krainc, D., Luquet, S., Sweet, I.R., Schwartz, M.W. and La Spada, A.R. (2006) *Cell Metab.*, **4**, 349-362.
[160] Underwood, B.R., Broadhurst, D., Dunn, W.B., Ellis, D.I., Michell, A.W., Vacher, C., Mosedale, D.E., Kell, D. B., Barker, R.A., Grainger, D.J. and Rubinsztein, D.C. (2006) *Brain*, **129**, 877-886.
[161] Mochel, F., Charles, P., Seguin, F., Barritault, J., Coussieu, C., Perin, L., Le Bouc, Y., Gervais, C., Carcelain, G., Vassault, A., Feingold, J., Rabier, D. and Durr, A. (2007) *PLoS ONE*, **2**, e647.
[162] Griffin, J.L., Cemal, C.K. and Pook, M.A. (2004) *Physiol. Genomics*, **16**, 334-340.
[163] La Spada, A.R., Wilson, E.M., Lubahn, D.B., Harding, A.E. and Fischbeck, K.H. (1991) *Nature*, **352**, 77-79.
[164] Bennett, E.J., Shaler, T.A., Woodman, B., Ryu, K.Y., Zaitseva, T.S., Becker, C. H., Bates, G. P., Schulman, H. and Kopito, R. R. (2007) *Nature*, **448**, 704-708.
[165] Fleming, T.R. and Demets, D.L. (1996) *Ann. Intern. Med.*, **125**, 605-613.

Received: November 18, 2007 Revised: December 31, 2007 Accepted: January 22, 2008