15. Van Camp G, Flamez A, Cosyns B, et al. Treatment of Parkinson's disease with pergolide and relation to restrictive valvular heart disease. Lancet 2004;363:1179–1183.
16. Van Camp G, Flamez A, Cosyns B, Goldstein J, Perdaens C, Schoors D. Heart valvular disease in patients with Parkinson's disease treated with high-dose pergolide. Neurology 2003;61:859–861.
17. Pritchett AM, Morrison JF, Edwards WD, Schaff HV, Connolly HM, Espinosa RE. Valvular heart disease in patients taking pergolide. Mayo Clin Proc 2002;77:1280–1286.
18. Flowers CM, Racoosin JA, Lu SL, Beitz JG. The US Food and Drug Administration's registry of patients with pergolide-associated valvular heart disease. Mayo Clin Proc 2003;78:730–731.
19. Weber M, Hamm C. Role of B-type natriuretic peptide (BNP) and NT-proBNP in clinical routine. Heart 2006; 92:843–849.
20. Detaint D, Messika-Zeitoun D, Chen HH, et al. Association of B-type natriuretic peptide activation to left ventricular end-systolic remodeling in organic and functional mitral regurgitation. Am J Cardiol 2006;97:1029–1034.
21. Eimer MJ, Ekery DL, Rigolin VH, Bonow RO, Carnethon MR, Cotts WG. Elevated B-type natriuretic peptide in asymptomatic men with chronic aortic regurgitation and preserved left ventricular systolic function. Am J Cardiol 2004;94:676–678.
22. Watanabe H, Atsuta N, Ito M, et al. Relationship between non-motor function and quality of life in Parkinson's disease; longitudinal study. Rinsho Shinkeigaku 2007;47: 1015. Abstract.
23. Calne DB, Snow BJ, Lee C. Criteria for diagnosing Parkinson's disease. Ann Neurol 1992;32:S125–S127.
24. Junghanns S, Glöckler T, Reichmann H. Switching and combining of dopamine agonists. J Neurol 2004;251 suppl 6:VI/19–23.
25. Millan MJ, Maiofiss L, Cussac D, et al. Differential actions of antiparkinson agents at multiple classes of monoaminergic receptor: I: a multivariate analysis of the binding profiles of 14 drugs at 21 native and cloned human receptor subtypes. J Pharmacol Exp Ther 2002;303:791–804.
26. Jahnichen S, Horowski R, Pertz HH. Agonism at 5-HT2b receptor is not a class effect of the ergolines. Eur J Pharmacol 2005;513:225–229.
27. Zoghbi WA, Enriquez-Sarano M, Foster E, et al. Recommendations for evaluation of the severity of native valvular regurgitation with two-dimensional and Doppler echocardiography. J Am Soc Echocardiogr 2003;16:777–802.
28. McKee PA, Castelli WP, McNamara PM, et al. The natural history of congestive heart failure: the Framingham study. N Engl J Med 1971;285:1441–1446.
29. Ho KK, Anderson KM, Kannel WB, et al. Survival after the onset of congestive heart failure in Framingham Heart Study subjects. Circulation 1993;88:107–115.
30. Droogmans S, Franken PR, Garbar C, et al. In vivo model of drug-induced valvular heart disease in rats: pergolide-induced valvular heart disease demonstrated with echocardiography and correlation with pathology. Eur Heart J 2007;28:2156–2162.
31. Niinuma H, Nakamura M, Hiramori K. Plasma B-type natriuretic peptide measurement in a multiphasic health screening program. Cardiology 1998;90:89–94.
32. Nakamura M, Endo H, Nasu M, Arakawa N, Segawa T, Hiramori K. Value of plasma B type natriuretic peptide measurement for heart disease screening in a Japanese population. Heart 2002;87:131–135.
33. Redfield MM, Rodeheffer RJ, Jacobsen SJ, Mahoney DW, Bailey KR, Burnett JC Jr. Plasma brain natriuretic peptide concentration: impact of age and gender. J Am Coll Cardiol 2002;40:976–982.
34. Eguchi K, Kario K, Hoshide S, et al. Greater change of orthostatic blood pressure is related to silent cerebral infarct and cardiac overload in hypertensive subjects. Hypertens Res 2004;27:235–241.
35. Setola V, Hufeisen SJ, Grande-Allen KJ, et al. 3,4-Methylenedioxymethamphetamine (MDMA, "Ecstasy") induces fenfluramine-like proliferative actions on human cardiac valvular interstitial cells in vitro. Mol Pharmacol 2003;63:1223–1229.
36. Newman-Tancredi A, Cussac D, Quen-tric Y, et al. Differential actions of antiparkinson agents at multiple classes of monoaminergic receptor. III. Agonist and antagonist properties at serotonin, 5-HT(1) and 5-HT(2), receptor subtypes. J Pharmacol Exp Ther 2002;303:815–822.
37. Reichmann H, Bilsing A, Ehret R, et al. Ergoline and non-ergoline derivatives in the treatment of Parkinson's disease. J Neurol 2006;253 suppl 4:iv36–iv38.
38. Miller WL, Hartman KA, Burritt MF, et al. Serial biomarker measurements in ambulatory patients with chronic heart failure: the importance of change over time. Circulation 2007;116:249–257.
39. Detaint D, Messika-Zeitoun D, Avierinos JF, et al. B-type natriuretic peptide in organic mitral regurgitation: determinants and impact on outcome. Circulation 2005;111: 2391–2397.

ABSTRACT: Spinal and bulbar muscular atrophy (SBMA) is an adult-onset motor neuron disease caused by a CAG repeat expansion in the androgen receptor gene. Because the progression of SBMA is slow, it is plausible to identify biomarkers that monitor disease course for therapeutic development. To verify whether the 6-min walk test (6MWT) is a biomarker of SBMA, we performed the 6MWT in 35 genetically confirmed patients and in 29 age-matched healthy controls. The walk distance covered within 6 min (6MWD) was significantly less in SBMA than it was in controls (323.3 ± 143.9 m and 637.6 ± 94.2 m, respectively; $P < 0.001$). In test–retest analysis, the intraclass correlation coefficient for the 6MWD was high in SBMA patients ($r = 0.982$). In a 1-year follow-up the 6MWD significantly decreased at a rate of 11.3% per year. Our observations suggest that the 6MWT is a biomarker that can be used to monitor progression of motor impairment in SBMA.

Muscle Nerve **38:** 964–971, 2008

WALKING CAPACITY EVALUATED BY THE 6-MINUTE WALK TEST IN SPINAL AND BULBAR MUSCULAR ATROPHY

YU TAKEUCHI, MD,[1] MASAHISA KATSUNO, MD, PhD,[1,2] HARUHIKO BANNO, MD, PhD,[1] KEISUKE SUZUKI, MD, PhD,[1] MOTOSHI KAWASHIMA, MD,[1] NAOKI ATSUTA, MD, PhD,[1] MIZUKI ITO, MD, PhD,[1] HIROHISA WATANABE, MD, PhD,[1] FUMIAKI TANAKA, MD, PhD,[1] and GEN SOBUE, MD, PhD[1]

[1] Department of Neurology, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan
[2] Institute for Advanced Research, Nagoya University, Nagoya, Japan

Accepted 12 May 2008

Spinal and bulbar muscular atrophy (SBMA) is a hereditary lower motor neuron disease that affects adult males exclusively. It has a prevalence of 1–2 per 100,000 of the total population.[13,20,28] The cause of SBMA is an aberrant elongation of a CAG repeat in the androgen receptor (AR) gene. CAG repeats range from 9 to 36 in normal subjects, but 38 to 62 repeats are found in SBMA patients.[3,22,31,32] CAG repeat expansion has also been detected in Huntington's disease and several forms of spinocerebellar ataxia.[14] The main symptoms of SBMA are weakness and atrophy of the bulbar, facial, and limb muscles. The onset of weakness is usually between 30 and 60 years, followed by slow progression of neuromuscular symptoms.[5] The onset of symptoms and clinical features of SBMA are dependent on the CAG repeat size, as has been observed in other polyglutamine diseases.[10,31]

Although there is no effective treatment for SBMA, several therapeutic candidates have recently emerged from studies in animal models, and clinical trials have been proposed.[18,19,24,33,36] It is, however, difficult to assess the effects of intervention on true disease endpoints such as the occurrence of pneumonia or the length of time a patient lives free from the use of a wheelchair, because the progression of symptoms is notably slow in SBMA.[5] Therefore, appropriate surrogate endpoints are needed to facilitate the clinical application of animal study results. In this regard, it is important to identify biomarkers of SBMA that reflect the pathogenic processes and can be used as surrogate endpoints. Although nuclear accumulation of mutant AR protein in the scrotal skin has been shown to be a candidate for a histopathological biomarker,[1,6] clinical parameters to evaluate motor function have not been established for SBMA.

The 6 min walk test (6MWT) is one of the most popular clinical tests used for assessment of functional capacity. This test evaluates the global and integrated responses of all the systems involved in walking, including the pulmonary and cardiovascular systems, neuromuscular units, muscle metabo-

Abbreviations: 6MWD, 6-min walk distance; 6MWT, 6-min walk test; ALS, amyotrophic lateral sclerosis; ALSFRS-R, ALS functional rating scale-revised; AR, androgen receptor; PCR, polymerase chain reaction; SBMA, spinal and bulbar muscular atrophy

Key words: spinal and bulbar muscular atrophy; 6-minute walk test; biomarker; exercise test; 6-minute walk duration

Correspondence to: G. Sobue; e-mail: sobuegi@med.nagoya-u.ac.jp or M. Katsuno; e-mail: ka2no@med.nagoya-u.ac.jp

Published online 21 July 2008 in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/mus.21077

lism, systemic circulation, peripheral circulation, and blood condition. Because the 6MWT accurately reflects patients' activities of daily living, it has been applied widely for evaluation of functional exercise capacity in cardiopulmonary disorders.[29] It has also been employed to assess functional exercise capacity in neuromuscular diseases such as stroke, Parkinson's disease, cerebral palsy, chronic poliomyelitis, multiple sclerosis, myotonic dystrophy, fibromyalgia, and spinal cord injury.[2,7,8,11,12,16,21,23,25,27] In diseases that affect multiple regions of the nervous system, the 6 min walk distance (6MWD) correlates well with other measurements that evaluate systemic motor function such as muscle strength, clinical scores, and health status questionnaires.[16,23,25]

The aim of this study was to evaluate functional exercise capacity in patients with SBMA using the 6MWT. We also investigated the natural history of the 6MWD in order to determine whether it is an appropriate biomarker that can be used as a surrogate endpoint in forthcoming clinical trials.

MATERIALS AND METHODS

Participants. A total of 35 patients with a diagnosis of SBMA confirmed by genetic analysis were included. Patients were included if they were capable of walking independently along a flat corridor with or without the use of a cane or similar equipment. Exclusion criteria included unstable angina or myocardial infarction during the previous month, tachycardia (>120/min), and uncontrolled hypertension (>180/100 mmHg). We also evaluated 29 age-matched control subjects in this study. The first test was performed between May 2006 and June 2007; reevaluation of 24 of the 35 SBMA patients was conducted ≈1 year after the initial test. The remaining cases were excluded from the follow-up evaluation, because they participated in another interventional study after their first 6MWT evaluation. The age-matched controls did not undergo serial testing.

In addition to the 6MWT, we also evaluated general motor function using clinical scales for amyotrophic lateral sclerosis (ALS), such as the Limb Norris Score, the Norris Bulbar Score, the ALS functional rating scale-revised (ALSFRS-R), and grip power. We defined the onset of disease as the time when muscle weakness began, but not when tremor of the fingers appeared. All studies conformed to the ethics guidelines for human genome/gene analysis research and the ethics guidelines for epidemiological studies endorsed by the Japanese government. The Institutional Review Board of Nagoya University Graduate School of Medicine approved the study, and all SBMA patients and normal subjects gave their informed consent for the investigation.

Six-min Walk Test. The 6MWT was performed according to the guidelines provided by the American Thoracic Society.[4] Briefly, examiners instructed participants to walk at their own pace as far as possible in 6 min. The patients were allowed to rest when needed. No encouragement was made throughout the test. The total distance walked during 6 min (6-min walk distance: 6MWD) was recorded. Patients with severe weakness were permitted to use a cane or equivalent assistive device if needed. The 6MWT was performed along a long, flat, straight, enclosed corridor with turnaround points at an interval of 30 m. Although the time of day was not the same, all tests were performed indoors with the same lighting and temperature. All patients were instructed to wear sneakers or equivalent shoes. The results were based on a single 6MWT. We did not perform repeated tests or practice sessions, because patients with SBMA have severe fatigability, which might produce unstable data and cause safety problems.[26,30,35] In addition to the 6MWD, we also recorded the Borg scale before and after the 6MWT.

To assess reliability the 6MWT was repeated within 60 days after the first test in 15 patients without informing them of the results of the previous test. Likewise, patients were not allowed to know the distance covered in the first test when they took the 1-year follow-up study.

Genetic Analysis. Genomic DNA was extracted from peripheral blood of SBMA patients using conventional techniques.[32] Polymerase chain reaction (PCR) amplification of the CAG repeat in the AR gene was performed using a fluorescein-labeled forward primer (5′-TCCAGAATCTGTTCCAGAGCGTGC-3′) and a nonlabeled reverse primer (5′-TGGCCTCGCTCAGGATGTCTTTAAG-3′). Detailed PCR conditions and measurement of CAG-repeat size were described previously.[10,32]

Data Analysis. All data are presented as means ± SD. Changes in the 6MWD were compared using a paired *t*-test. Correlations among the parameters were analyzed using Pearson's correlation coefficient. *P*values less than 0.05 and correlation coefficients (r) greater than 0.4 were considered to indicate significance. Calculations were performed using the statistical software package SPSS 14.0J (SPSS Japan, Tokyo, Japan).

RESULTS

Clinical and Genetic Backgrounds of SBMA Patients. The clinical characteristics of the study population are presented in Table 1A. There were a total of 35 subjects in the study. All participants were male and of Japanese nationality. The duration from onset was assessed at the first notice of motor impairment[5] and this ranged from 1 to 32 years. There was no significant difference between the median CAG repeat length in the present study and those reported previously in SBMA patients.[3,22,32] All patients were ambulatory with or without aid, and none were bedridden or wheelchair-bound. Other complications that required medication were found in 33 (94.3%) out of 35 patients: diabetes mellitus in 12 (34.3%), hyperlipidemia in 23 (65.7%), hypertension in 20 (57.1%), and depression in 3 (8.6%). Ischemic heart disease or pulmonary disorders were not documented in any patients included in this study.

Reliability of the 6MWT in SBMA Patients. To estimate test–retest reliability we performed the 6MWT on 15 randomly chosen SBMA patients on two different occasions at an interval of 29.4 ± 10.9 days. There was no statistical difference between the clinical scores of the patients who underwent test–retest analysis and those of the remaining subjects (Table 1B). In each patient the two tests were conducted by different examiners. As shown in Figure 1, when the two sets of the 6MWT were compared with one another the intraclass correlation coefficient was 0.982 ($P < 0.001$), indicating an excellent test–retest reliability for SBMA patients.

6MWD and Relevant Clinical Parameters in SBMA. To verify that the 6MWT detects motor impairment in SBMA patients we compared the data from a total of 35 cases and 29 age-matched control subjects (Table 2). There was a significant difference ($P < 0.001$) in

Table 1. Clinical and genetic features of SBMA patients.

A Clinical and genetic features	Mean ± SD (range)	*n*
Age at examination (years)	55.8 ± 11.2 (33–74)	35
CAG repeat length in AR gene (number)	48.3 ± 3.5 (42–57)	29*
Duration from onset (years)	9.7 ± 7.1 (1–32)	35
Limb Norris Score (normal score = 63)	52.5 ± 7.3 (34–62)	35
Norris Bulbar Score (normal score = 39)	33.3 ± 4.2 (20–39)	35
ALSFRS-R (normal score = 48)	41.2 ± 3.7 (33–47)	35
Grip power (kg)†	18.7 ± 5.3 (8.3–33.9)	33

B	Followed-up group		Non followed-up group		
Clinical and genetic features	Mean ± SD (range)	*n*	Mean ± SD (range)	*n*	*P*
Age at examination (years)	55.3 ± 10.9 (33–70)	24	56.7 ± 12.3 (34–74)	11	NS
CAG repeat length in AR gene (number)	48.6 ± 3.6 (42–57)	19*	47.7 ± 3.4 (42–52)	10*	NS
Duration from onset (years)	10.3 ± 7.7 (1–32)	24	9.4 ± 5.9 (2–21)	11	NS
Limb Norris Score (normal score = 63)	52.3 ± 7.3 (34–62)	24	52.9 ± 7.7 (39–62)	11	NS
Norris Bulbar Score (normal score = 39)	33.3 ± 4.3 (20–39)	24	33.5 ± 4.1 (25–38)	11	NS
ALSFRS-R (normal score = 48)	41.0 ± 3.5 (35–46)	24	41.5 ± 4.2 (33–47)	11	NS
Grip power (kg) †	19.6 ± 4.3 (12.0–27.2)	22	17.0 ± 6.7 (8.3–33.9)	11	NS

C	Retested group		Non retested group		
Clinical and genetic features	Mean ± SD (range)	*n*	Mean ± SD (range)	*n*	*P*
Age at examination (years)	57.0 ± 11.7 (34–74)	15	54.9 ± 11.0 (33–68)	20	NS
CAG repeat length in AR gene (number)	48.4 ± 3.9 (42–57)	14*	48.3 ± 3.2 (42–53)	15*	NS
Duration from onset (years)	9.3 ± 5.6 (2–21)	15	10.5 ± 8.2 (1–32)	20	NS
Limb Norris Score (normal score = 63)	52.7 ± 7.4 (39–62)	15	52.3 ± 7.5 (34–62)	20	NS
Norris Bulbar Score (normal score = 39)	32.7 ± 5.1 (20–38)	15	33.8 ± 3.4 (26–39)	20	NS
ALSFRS-R (normal score = 48)	41.4 ± 4.1 (33–47)	15	41.0 ± 3.4 (35–46)	20	NS
Grip power (kg) †	18.4 ± 6.3 (8.3–33.9)	15	19.0 ± 4.4 (12.0–27.2)	18	NS

Data are shown as mean ± SD.

**The abnormal elongation of the CAG repeat was confirmed by gene analysis using agarose gel electrophoresis without determining the repeat number in the remaining 6 patients.*

†The average of both hands. The normal control data of male Japanese at 50–54 years: 43.7 ± 6.4 kg. (The report of physical strength and athletic capability surveillance in 2005.)

AR, androgen receptor; ALSFRS-R, ALS functional rating scale-revised; NS, not significant.

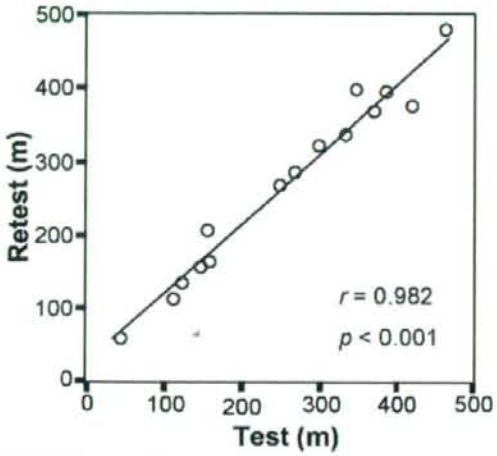

FIGURE 1. Test–retest analysis of the distance walked within 6 min (6MWD). Two sets of 6-min walk tests were carried out within 60 days in 15 SBMA patients who did not know the result of their previous test.

the 6MWD between SBMA patients and the controls. There was no significant difference in the posttest Borg scale, a semiquantitative measure of perceived exercise-related fatigue, between SBMA patients and controls.

We also evaluated motor function of SBMA patients using functional scales and grip power. Because there are no specific motor scores for SBMA we adopted the functional scales used for ALS. The values of the 6MWD correlated well with those of the Limb Norris Score, the Norris Bulbar Score, and the ALSFRS-R (Fig. 2A–C). The value of the 6MWD was inversely correlated with disease duration, although there was no correlation between the 6MWD and grip power (Fig. 2D,E).

Natural History of the 6MWD in SBMA. To delineate the progression rate of walking disturbance the 6MWT was reperformed in a group of 24 SBMA patients at 54.3 ± 6.8 weeks after the initial evaluation. Although follow-up data for the remaining cases was not available because of participation in another interventional study, there was no statistical difference between the backgrounds of the followed subjects and the remaining cases (Table 1C). The 6MWD was significantly decreased in comparison with the distance in the first test ($P = 0.001$), although no motor functional scales showed significant deterioration during the same time period (Table 3). It should be noted that the rate of decline was 11.3 ± 17.6%, which was relatively constant regardless of the walking capacity at the initial evaluation (Fig. 3A). When the patients were stratified by their baseline severity, the change in 6MWD during the follow-up period was larger in the less affected subgroup (Fig. 3B,C).

Based on the natural history of the 6MWD in SBMA, we calculated the sample size for a clinical trial targeting 6MWD, the Limb Norris Score, the Norris Bulbar Score, and ALSFRS-R (Table 4). The sample sizes for a clinical trial using the 6MWT are smaller than those using motor scales, suggesting that it is a more feasible outcome measure than the other clinical scores.

Safety of the 6MWT in SBMA Patients. Throughout the tests, no adverse events such as angina and dyspnea were reported. Although 10 cases walked using a cane and 1 patient walked leaning against a wall, no patients fell or tripped during the tests.

DISCUSSION

This study demonstrates that the 6MWT is a practical, reliable, and safe procedure to measure the walking capacity of SBMA patients. Gait disturbance is the initial symptom in the majority of SBMA patients, and it precedes other health problems such as respiratory failure and dysphagia by ≈10–20 years.[5] Therefore, walking capacity is one of the strongest determining factors of activities of daily living during disease progression in SBMA, implying that the 6MWT appears to be a valuable target for future therapeutic interventions. Although the duration of illness of the patients we tested ranged from 1 to 32 years, our observations suggest that the 6MWT is applicable for patients with various disease durations as long as they are capable of walking.

As a quantitative measure of walking capacity the 6MWT was originally developed for cardiorespiratory and cardiovascular populations. Since then, this test has been applied to various medical conditions including neuromuscular disorders. For example, the 6MWD is strongly correlated with functional

Table 2. Six-min walk distance (6MWD) in SBMA and healthy controls.

	SBMA ($n = 35$)	Healthy controls ($n = 29$)	P
6MWD (m)	323.3 ± 143.9	637.6 ± 94.2	< 0.001
Age at examination (years)	55.8 ± 11.2	52.8 ± 10.7	NS
Borg scale	3.9 ± 2.4	3.2 ± 2.1	NS

Data are shown as mean ± SD.

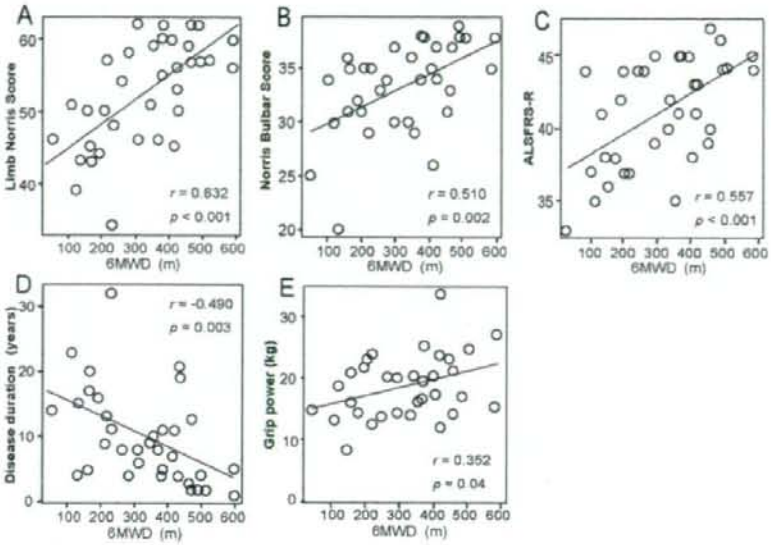

FIGURE 2. Correlation between the 6MWD and other measurements of motor function. **A–C:** The correlation between the 6MWD and general motor function. There was a significant correlation between the 6MWD and motor functional scales such as the Limb Norris Score **(A)**, the Norris Bulbar Score **(B)**, and the ALS functional rating scale-revised (ALSFRS-R, **C**). **D:** The value of the 6MWD was inversely correlated with disease duration. **E:** There was no correlation between the 6MWD and grip power. The value of grip power is shown as the average of left and right hands. 6MWD, six-min walk distance.

scores for balance, strength, and spasticity in post-stroke patients.[12] In patients with multiple sclerosis, the 6MWD is shortened when compared to age-matched healthy controls, reflecting physical function disability.[27] Moreover, the 6MWT has been used as an outcome measurement in various clinical trials for patients with mucopolysaccharidosis, postpolio syndrome, and stroke.[9,15,17,34] Partially due to the small number of patients, there are no established clinical parameters to quantify motor function in SBMA. The total distance covered in 6 min correlated well with motor functional scores in SBMA patients in the present study. Because SBMA is a single gene disorder, various tissues are affected to a similar extent in this disease.[6] This appears to be the reason why 6MWD correlates excellently with clinical scores that reflect disability of other parts of the nervous system affected in SBMA. In addition to muscle weakness, SBMA patients often perceive fatigue during continuous exercise, suggesting that objective measurement to detect the degree of functional endurance is feasible for this disease. Our findings indicate that the 6MWT is a practical examination for measuring functional exercise capacity of patients with SBMA and those with other neurodegenerative diseases.

Table 3. Chronological change in motor function in SBMA.

	n	Initial test	Follow-up	*P*
6MWD (m)	24	351.0 ± 142.8	308.5 ± 132.0	0.001
ALSFRS-R	24	41.0 ± 3.5	39.8 ± 4.0	NS
Limb Norris Score	24	52.3 ± 7.3	50.9 ± 8.2	NS
Norris Bulbar Score	24	33.3 ± 4.3	32.9 ± 4.4	NS

Data are shown as mean ± SD. ALSFRS-R, ALS functional rating scale-revised.

Repeated measurement procedures might result in misleading results, because of practice effect. Therefore, it is important to investigate the reliability of this test in order to determine its feasibility for clinical measurement. Although the 6MWD has been shown to increase only slightly in a second performance a day later, the effect of practice wears off after a week.[4,21] The present study also demonstrated that the reliability of the 6MWT is excellent for SBMA patients, if they are tested within an interval of ≈1 month.

Given the rapid advance of therapeutic developments in animal studies, it is a high priority to search for biomarkers to determine disease severity and

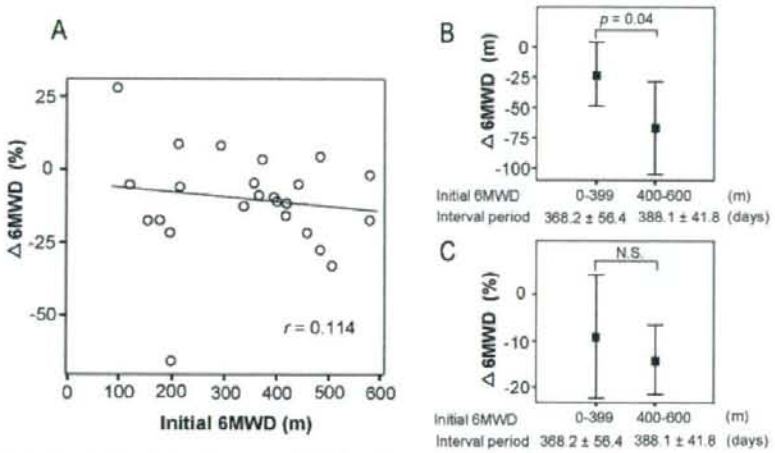

FIGURE 3. The relationship between the change in the 6MWD and the distance in the initial test. **A:** The decline in the 6MWD did not correlate with the result of the initial evaluation. **B,C:** The decline in the 6MWD for each of the severity groups (**B,** actual change; **C,** % change). There was no statistical difference in the follow-up period between both groups.

progression in SBMA. A biomarker is an objectively measurable parameter that indicates the pathogenic process and potentially serves as a surrogate endpoint in a treatment trial. Candidates for biomarkers in neurodegenerative diseases include: motor functional scales; serological parameters; electrophysiological data; histopathological findings; and neuroimaging parameters. It is feasible to analyze combinations of biomarkers to monitor disease progression. There are an increasing number of biomarkers for Alzheimer's disease, but there is a paucity of biomarkers identified in other neurodegenerative diseases. In fact, identification of biomarkers has been hampered by small numbers of patients, slow disease progression, and lack of objective clinical measurements for SBMA. In the present study the 6MWD was significantly decreased in patients with SBMA compared with age-matched healthy subjects, and it correlated well with other scores that measure general motor function. This suggests that the test is capable of detecting motor impairment in SBMA patients. Furthermore, our longitudinal analysis showed that the 6MWD decreases by ≈10% per year in SBMA patients despite no detectable deterioration in the other motor functional parameters we examined. According to our sample size calculation, the number required for a clinical trial appears to be reduced to one-fifth by changing the endpoint from motor scores to 6MWD. For example, when the therapeutic effect is estimated to be 50%, a trial targeting ALSFRS-R needs 500 patients, but the size is diminished to 100 by adopting 6MWD as the endpoint (Table 4). Although it is a limitation of this study that the follow-up data for 11 out of 35 cases was not obtained, there was no statistical difference between the backgrounds of the followed subjects and the remaining cases. This suggests that the 24 patients who underwent the follow-up study represent the whole group. Because the interval between the onset of weakness and the need for a wheelchair has been reported to be ≈15 years, the observed rate of decrease in the 6MWD is likely reasonable.[5] Therefore, our findings also suggest that the 6MWT is an indicator of disease progression, which can be used as an outcome measurement in future clinical trials for SBMA. Our observation that the annual change in 6MWD is influenced by the disease severity might suggest the need for stratification in the design of clinical trials. Given that scrotal skin biopsy analysis is

Table 4. Sample size calculation.

	Estimated therapeutic effect (%)	
Outcome measure	50	70
6MWD	102	52
ALSFRS-R	508	259
Limb Norris Score	462	236
Norris Bulbar Score	2,650	1,352

Data are shown as the number of patients per group (P = 0.05, power = 0.8).
ALSFRS-R, ALS functional rating scale-revised.

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

M Iijima,[1] H Koike,[1] N Hattori,[1] A Tamakoshi,[2] M Katsuno,[2] F Tanaka,[1] M Yamamoto,[1] K Arimura,[3] G Sobue,[1] the Refractory Peripheral Neuropathy Study Group of Japan

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

[1] Department of Neurology, Nagoya University Graduate School of Medicine, Nagoya, Japan; [2] Division of Clinical Trials, National Centre for Geriatrics and Gerontology, Aichi, Japan; [3] Department of Neurology and Geriatrics, Kagoshima, University Graduate School of Medical and Dental Sciences, Kagoshima University, Kagoshima, Japan

Correspondence to: Professor G Sobue, Department of Neurology, Ngoya, University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, 466-8550 Nagoya, Japan; sobueg@med.nagoya-u.ac.jp

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

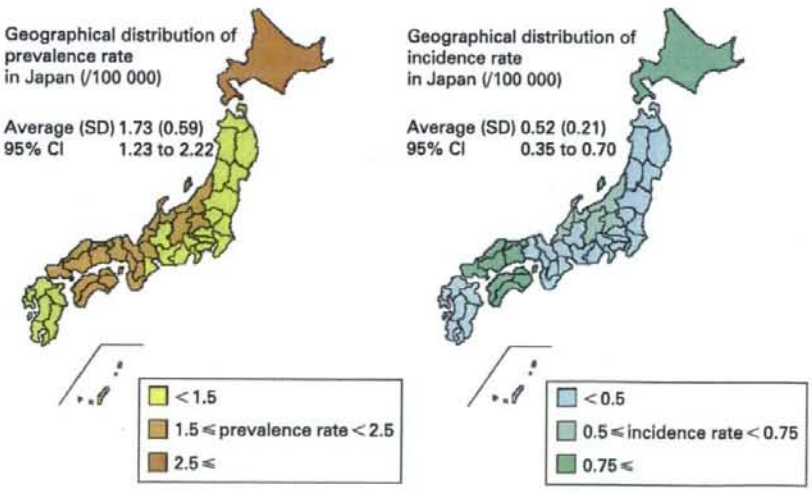

Figure 1 Geographic distribution of prevalence and incidence rates throughout Japan. There were no statistical preponderances in the geographical distributions of either the prevalence or incidence rates.

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[15] (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[13] (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

Members of the Refractory Peripheral Neuropathy Research Study Group of Japan

S Yagihashi, Department of Pathology, Hirosaki University School of Medicine, Hirosaki, Japan; T Yamamura, Department of Immunology, National Institute of Neuroscience, NCNP, Kodaira, Japan; S Ikeda, Department of Neurology, Shinshu University School of Medicine, Matsumoto, Japan; M Nakagawa, Department of Pathology and Applied Neurobiology, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Kyoto, Japan; S Kusunoki, Department of Neurology, Kinki University School of Medicine, Osaka, Japan; K Inoue, Department of Mental Retardation and Birth Defect Research, National Centre of Neurology and Psychiatry, Kodaira, Japan; K Hayasaka, Department of Pediatrics, Yamagata University School of Medicine, Yamagata, Japan; K Matsumura, Department of Neurology and Neuroscience, Teikyo University School of Medicine, Tokyo, Japan; Y Ando, Department of Cell Pathology, Graduate School of Medical and Pharmaceutical Sciences, Kumamoto University, Kumamoto, Japan; M Baba, Department of Neurology, Hirosaki University School of Medicine, Hirosaki, Japan; M Nakazato, Department of Neurology, Respirology, Endocrinology and Metabolism, Internal Medicine, Faculty of Medicine, University of Miyazaki, Miyazaki, Japan; H Yasuda, Division of Neurology, Department of Medicine, Shiga University of Medical Science, Shiga, Japan; R Kaji, Department of Neurology, University of Tokushima, Faculty of Medicine, Tokushima, Japan; O Onodera, Department of Neurology, Clinical Neuroscience Branch, Niigata University Brain Research Institute, Niigata, Japan; J Kira, Department of Neurology, Neurological Institute, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan; S Kuwabara, Department of Neurology, Chiba University Graduate School of Medicine, Chiba, Japan; K Arimura, Department of Neurology and Geriatrics, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima University, Kagoshima, Japan; G Sobue, Department of Neurology, Nagoya University Graduate School of Medicine, Nagoya, Japan.

Molecular Genetics and Biomarkers of Polyglutamine Diseases

Masahisa Katsuno*[,1,2], Haruhiko Banno[2], Keisuke Suzuki[2], Yu Takeuchi[2], Motoshi Kawashima[2], Fumiaki Tanaka[2], Hiroaki Adachi[2] and Gen Sobue*[,2]

[1]*Institute for Advanced Research, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-8601, Japan*

[2]*Department of Neurology, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan*

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.

*Address correspondence to these authors at the Institute for Advanced Research, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-8601, Japan; Tel: +81-52-744-2391; Fax: +81-52-744-2394;
E-mail: ka2no@med.nagoya-u.ac.jp

Department of Neurology, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan; Tel: +81-52-744-2385; Fax: +81-52-744-2384;
E-mail: sobueg@med.nagoya-u.ac.jp

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

1566-5240/08 $55.00+.00

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

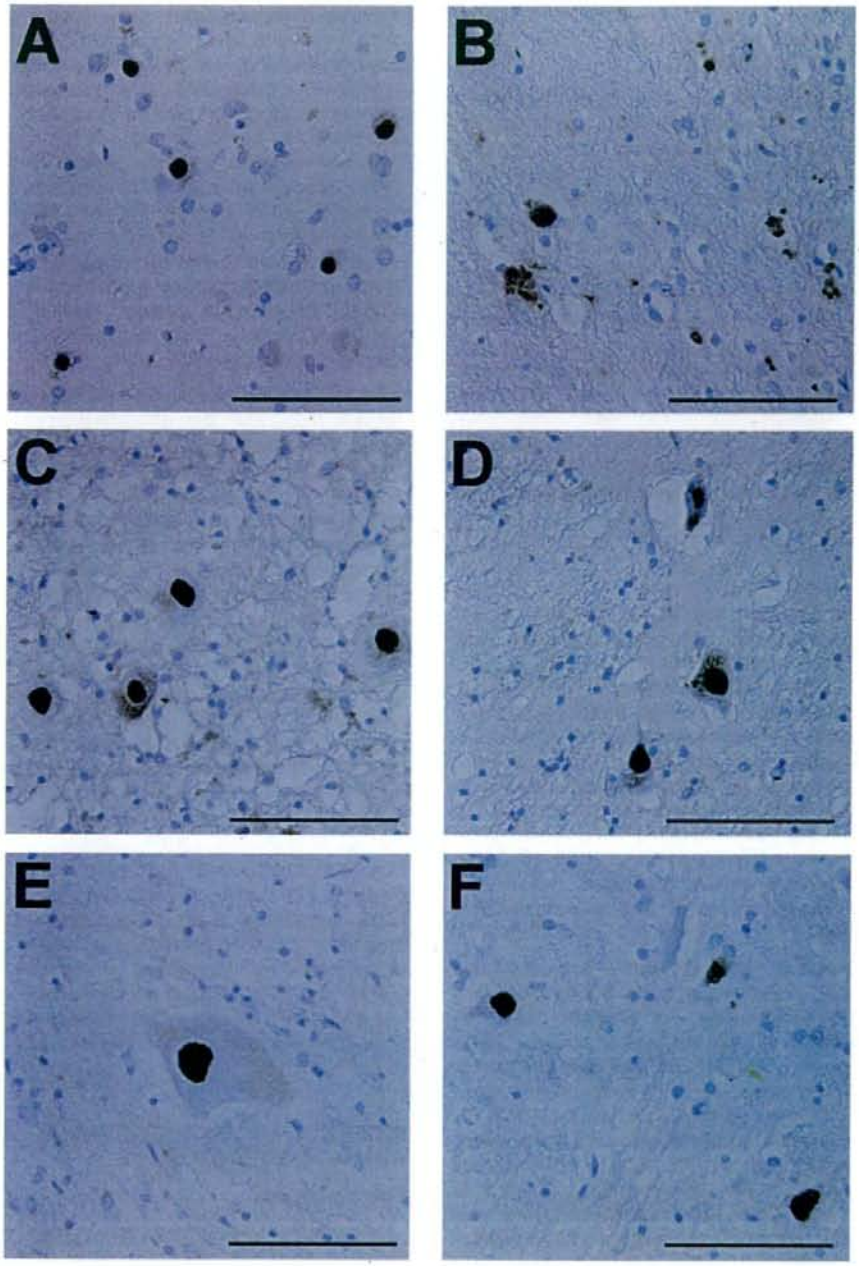

Fig. (1). Accumulation of abnormal proteins in polyglutamine diseases. Immunohistochemistry of autopsy specimens from patients using an anti-polyglutamine antibody (1C2). A. Cerebral cortex, HD; B. Putamen, HD; C. Dentate nucleus, DRPLA, D. globus pallidus, DRPLA; E. Anterior horn of spinal cord, SBMA;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

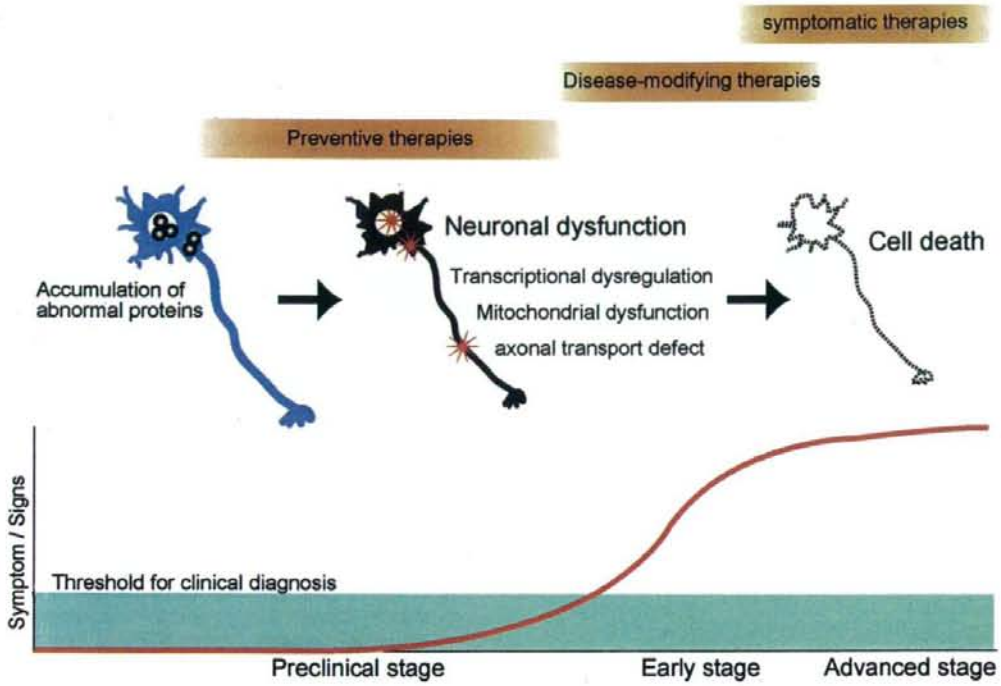

Fig. (2). Pathogenesis of polyglutamine diseases and the role of biomarkers. Accumulation of causative proteins, the culprit of neurodegeneration, triggers several downstream events in the pathogenesis of polyglutamine diseases: dysregulation of transcription, mitochondrial dysfunction and disruption 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].

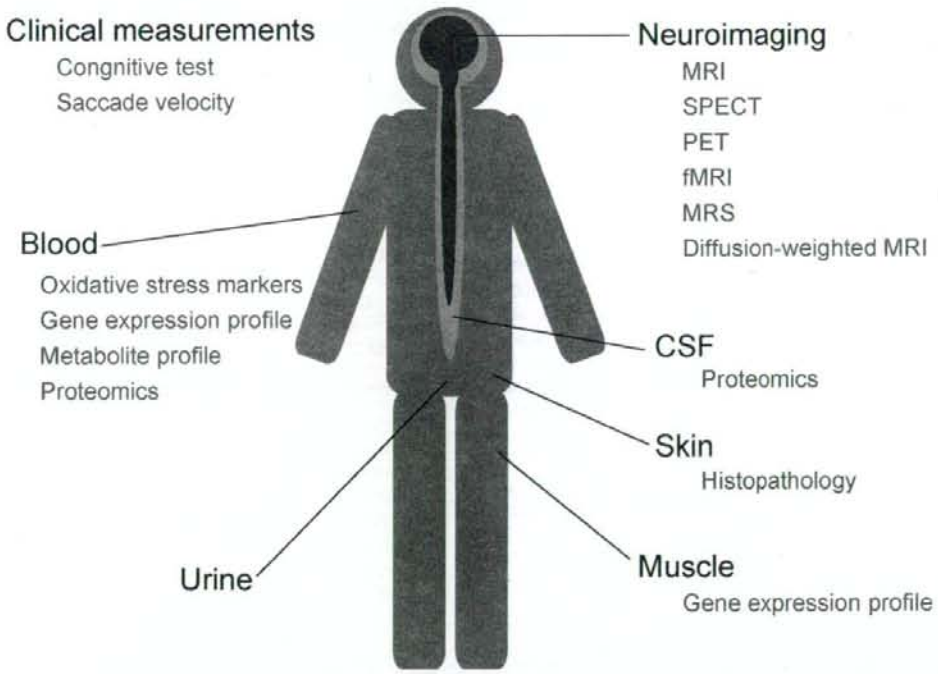

Fig. (3). Potential biomarkers for polyglutamine diseases. Animal and clinical studies have provided candidate biomarkers for polyglutamine diseases, including: neuroimaging; biofluid analysis; clinical measurement; gene expression profiles; proteomics; metabonomic analysis and histopathology; some of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